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[Continued on next page]

(54) **Title:** OSTEOCHONDRORETICULAR STEM CELLS FOR BONE AND CARTILAGE REGENERATION

(57) **Abstract:** The invention is directed to osteochondroreticular stem cells and methods of using osteochondroreticular stem cells. In another aspect the invention is directed to a method of treating osteoarthritis or skeletal fractures using osteochondroreticular stem cells.

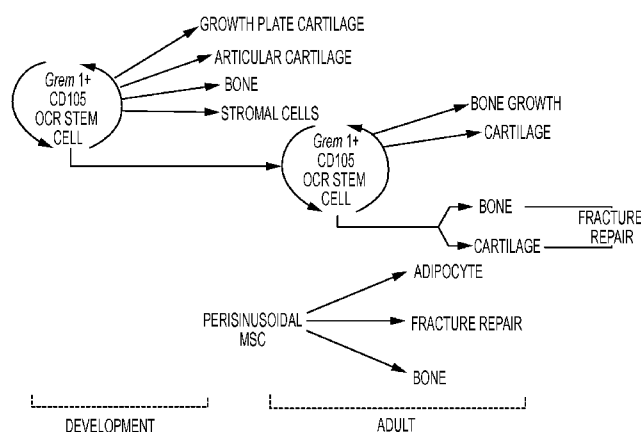


FIG. 7

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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OSTEOCHONDRORETICULAR STEM CELLS FOR BONE AND CARTILAGE REGENERATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/017,664, entitled, "Osteochondroreticular stem cells for bone and cartilage regeneration" filed on November 7, 2014, the contents of which are incorporated herein.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under RHL115145A and NIH5U54CA126513 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Arthritis sufferers include men and women, children and adults. Approximately 350 million people worldwide have arthritis. Nearly 40 million persons in the United States are affected by arthritis, including over a quarter million children. More than 21 million Americans have osteoarthritis. Cartilage and bone deterioration are a common consequence of aging, but poor diet, sedentary lifestyle, excess weight or injury can also result in damaged tissue. Mature cartilage is avascular and doesn't heal well after injury. Surgery is one option for repairing or replacing a damaged joint, but the costs are high and there are also several risks involved in the procedure, such as rejection in the event of joint replacement and infection.

[0004] Osteoarthritis describes a disease involving the degeneration in the protective cartilage around bones and occurs to millions of patients worldwide. Osteoarthritis is just one example of cartilage and bone deterioration and is a debilitating disease that affects millions of people. With the aging population, it is expected to continue to be a major disease worldwide. When the protective cartilage wears down, bones can rub together under joint movement, causing pain and stiffness, which may lead to decreased movement and morbidity. Current treatments include non-steroidal anti-inflammatories that can pose cardiovascular and gastrointestinal side effects, narcotics, and physical therapy to alleviate the symptoms. More invasive joint replacement

surgeries are needed for severe cases. No cure exists at the moment other than replacement of the joint.

[0005] The present invention provides an alternative approach to treatment.

SUMMARY

[0006] Inventive embodiments herein are based on the discovery of a newly identified stem cell, the osteochondroreticular (OCR) stem cell that is shown to be the chief origin of cartilage and bone during development. Certain conventional therapies to address bone and cartilage degeneration and injury have utilized pooled mesenchymal stem cells. The novel OCR stem cell population described herein provides significantly improved therapeutic methods and/or implantable products, compared to conventional pooled mesenchymal stem cell populations, particularly for the repair of cartilage due to their superior chondrogenic properties.

[0007] Certain embodiments provide improved stem cell therapy methods of treating diseases, degeneration or injury of the bone and cartilage as described herein including but not limited to osteoarthritis, osteoporosis, and bone fractures using OCR stem cells.

[0008] It has been determined that OCR stem cells contribute temporally and make lineage-specific contributions to skeletal development and maintenance. These stem cells are isolated from mesenchymal stromal cells found typically in the bone and bone marrow and can be distinguished by expression of Gremlin 1 (*Grem1*) and/or cell surface markers such as CD200, CD109 and CD105, markers identified through microarray screens. In addition, OCR stem cells are more clonogenic than other mesenchymal stem cells. OCR stem cells are lineage restricted skeletal stem cells that are determined in their skeletal fate and do not give rise to muscle and fat.

[0009] Embodiments of the invention also include a method for isolating OCR stem cells. Isolated OCR stem cells are useful in regenerating cartilage or bone tissue. Particularly, OCR stem cells are useful for treatment of diseases, degeneration or injury of the bone and cartilage resulting from age, gender, genes, excess weight, poor diet, sedentary lifestyle, injury or trauma,, abnormal metabolism (such as gout and pseudogout), osteoarthritis, infections (such as in the arthritis of Lyme disease), and an overactive immune system (such as rheumatoid arthritis and systemic lupus erythematosus).

[0010] A significant percentage but not all of the OCR stem cells express CD105, a well-established marker of bone marrow, (*Grem1*+ CD105 OCR stem cells) and have skeletal tissue fates. Therefore, in certain embodiments, the method comprises the steps of: (a) obtaining

multipotent mesenchymal stromal cells from a subject that comprise OCR stem cells and then isolating from the multipotent mesenchymal stromal cells a population of cells that express Gremlin 1 (Grem1) and/or by particular cell surface markers selected from the group consisting of CD200, CD109, and CD105; and (c) isolating OCR stem cells from the bone and/or bone marrow, wherein the isolated OCR stem cells promote regeneration of cartilage tissue and/or bone. In an alternative embodiment, the OCR stem cells may be isolated without necessarily utilizing cell markers, but can be identified retrospectively by their capacity to generate bone and cartilage *in vitro* (in culture), but poor efficiency for developing into fat cells.

[0011] In some embodiments, the isolated Grem1+ CD105 OCR stem cells are subjected to conditions that promote differentiation into osteoblasts, chondrocytes, and reticular marrow stromal cells that are useful for regeneration of cartilage tissue for treatment of diseases described herein. The conditions that promote differentiation comprise culturing the OCR stem cells in the presence of medium that comprises certain factors such as one or more bone morphogenic proteins (BMPs).

[0012] In addition, certain embodiments of the invention comprise a composition comprising an acceptable carrier and the isolated Grem1+ CD105 OCR stem cells described above in this paragraph and throughout the specification. Optionally, the composition may comprise other therapeutic agents. Certain embodiments of the invention also include substantially pure isolated Grem1+ CD105 OCR stem cells from tissue such as bone and bone marrow, which express the cell marker Grem1+ and CD105. In other specific embodiments, methods are provided for treating osteoarthritis and bone fracture by administering a therapeutically effective amount of the composition. The composition may be administered to tissue surround the fracture.

[0013] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures.

DESCRIPTION OF THE DRAWINGS

[0014] The following drawings form part of the present specification and are included to further demonstrate certain embodiments of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0015] **FIG. 1A-1K** illustrates that *Grem1* identifies rare adult multipotent mesenchymal stromal cells. (A) Protocol and (B) *Grem1-creERT^T; R26-LSL-TdTomato; Nes-GFP* mouse femur, showing that metaphyseal *Nes-GFP⁺* (green) and *Grem1⁺* cells (red, white arrows) are distinct. (C) Adult *Grem1-creERT^T; R26-LSL-TdTomato* bone marrow cells are rare and mesenchymal (CD45⁻CD31⁻Ter-119⁻). (D-G) Adult *Grem1-creERT^T; R26-LSL-TdTomato; Nes-GFP* mice: *Grem1* and mesenchymal *Nes-GFP* cells do not overlap, and clonogenicity is greater in the *Grem1⁺* versus the mesenchymal *Nes-GFP⁺* cells (CD45⁻CD31⁻Ter-119⁻). (E and F) 10 cm cell culture dish; (G) n = 5, data shown with mean ±SD, p = 0.013. (H-K) *Grem1⁺* cells from *Grem1-creERT^T; R26-LSL-ZsGreen; Acta2-RFP* mice could be clonally expanded in vitro and differentiated into bone (H) (alizarin red), cartilage (I) (toluidine blue), and myofibroblasts (J) (*Grem1⁺* green-derived cells with coexpression of *Acta2* [red]), but very limited adipogenesis (oil red) (K). Lower right insets show equivalent stain in a control marrow culture. In all graphs, the data are shown with the mean ±SD.

[0016] **FIG. 2A-2L** illustrates that *Grem1⁺* cells are enriched for CD105 bone marrow cells with upregulated osteochondral versus adipogenic gene expression (A-F) n = 3, *Grem1⁺* cells from adult, collagenase-digested whole bone and bone marrow were compared to the *Grem1*-negative population. On average, 40% (95% CI 20%–60%) of all *Grem1* cells were CD45⁻CD31⁻Ter-119⁻CD105⁺ compared to only 1.8% of *Grem1*-negative cells (F, p = 0.014). (C) *Grem1⁺* cells, however, were not further enriched for other MSC markers CD140a and Sca-1. *Grem1⁺* and *Grem1*-negative cells were compared across the increasingly specific immunophenotypes; data shown with mean ±SD. (G) Microarray was performed to compare the *Grem1⁺* (red) to the nonrecombined stromal (CD45⁻CD31⁻Ter-119⁻) population (green); in qPCR, we also sorted and evaluated the CD45⁻CD31⁻Ter-119⁻ population (blue) that did not

contain any recombined cells. (H) *Grem1*⁺ cells were not enriched for *Nes* expression. (I–L) qPCR confirmation of microarray revealed that *Grem1*⁺ cells had increased expression of pericytic (*Cpsg4*; I) and osteochondral genes (*Acan* and *Sp7*; J and K) but no association with the adipogenic differentiation gene *Pparg* (L). In all graphs, the data are shown with the mean $\pm$ SD.

[0017] **FIG. 3A–3P** illustrates that endogenous *Grem1* cells self-renew and lineage trace bone, cartilage, and stroma. (A) Protocol. (B–E) P1 induction in *Grem1-creERT*^T; *R26-LSL-ZsGreen*; *Acta2-RFP* mice. (B) At 24 hr after tamoxifen, *Grem1* recombined (green) only within the primary spongiosa of long bones distinct from the *Acta2-RFP* (red) cells in the marrow. But, over the following 96 hr (C), the *Grem1* cells began organizing into chondrocytic columns (D) and differentiated into stromal cells that invade the bone marrow (E) intertwined with *Acta2*-positive (red) cells. (F–I) *Grem1-creERT*^T; *R26-LSL-TdTomato*; *2.3colGFP* mice induced at P1, examined at 6 weeks, show that the *Grem1*⁺ cells generate reticular marrow stromal cells (G), chondrocytes in the epiphyseal plate (H), osteoblasts (*2.3colGFP*⁺, thus yellow) in the trabecular bone (I). *Grem1-creERT*^T; *R26-Confetti* P1 induction, examined at 6 weeks, revealed clonal populations of chondrocytes, and (K) serial sections confirm mixed clones, yellow clone shown, of chondrocytes and marrow stromal cells, low- and higher-power (inset) images. (L–P) Adult induction in *Grem1-creERT*^T; *R26-LSL-TdTomato* analyzed 11 months after adult induction (L). *Grem1*⁺ cells had generated chondrocytes (M and N), reticular marrow stromal cells (O), and bone and periosteal cells (red) (P).

[0018] **FIG. 4A–4H** illustrates that *Nes-GFP* cells make little contribution to skeletal tissues during early life. (A) Whole-mount in situ hybridization on mouse embryos at E9.5, E10.5, E11.5 and E12.5. These embryos were evaluated for *Sox9*, *Runx2*, *Grem1*, and *Nes* expression. *Nes-creERT*^T; *R26-LSL-TdTomato*; *Nes-GFP* mice (n = 3) were generated to lineage trace from *Nes-GFP*-positive cells throughout the bone marrow. These mice were induced at P1 and examined 6–8 weeks after. (B) Protocol. (C) By flow cytometry, approximately 4% of *Nes-GFP*-positive cells recombined (that is, were both green and red). (D–H) This specific *Nes-CreERT* transgenic line recombined in all typical *Nes-GFP* populations, including perivascular cells immediately inferior to the growth plate (D), in periarteriol cells (E), and in perisinusoidal

Nes-GFP-positive cells (red arrows, F–H). The only osteochondral lineage tracing found were isolated osteocytes throughout the diaphyseal bone (H, white arrow).

[0019] FIG. 5A-5L illustrates that adult *Grem1* cells, both endogenous and transplanted, differentiate into osteochondral fracture callus. (A) Protocol. (B and C) *Grem1-creERT^T; R26-LSL-TdTomato; 2.3colGFP* mice adult induction: *Grem1*⁺ (red) cells were not osteoblasts (green) but were adjacent to each other in situ (B) and during the first week of adherent bone marrow stromal culture (C). (D) is an X-ray of the femoral osteotomy and internal fixation of the bone. (E) and (F) show the serial histology and fluorescent microscopy sections from the resulting fracture callus after the osteotomy. (G) and (H) are magnified from areas shown in (F). *Grem1*⁺ cells (red) stream into the fracture site and differentiate into either osteoblasts (G, yellow cells, white arrows) or Sox9⁺ (white, nuclear stain) chondrocytes (yellow arrows) (H). (I) A *Grem1*⁺ clone, after adult induction, was expanded in vitro. An osteotomy with internal fixation was performed in wild-type mice, at which point 500 3 10⁶ clonal cells (red) were irrigated into the surgical field. Seven days later, the *Grem1* clone had engrafted (J), the site of injury was identified by TdTomato fluorescence imaging, and recombined cells (fluorescent red) differentiated into osteoblastic cells (K) (alkaline phosphatase positive cells, red-brown, white arrows) in the callus (sequential fluorescence microscopy and ALP staining performed on the same slide). (L) Callus culture was performed, and the recombined *Grem1* cells were easily recovered in vitro and serially transplanted into a secondary fracture (FIG. 12E).

[0020] FIG. 6A-6I illustrates that *Grem1* expression identifies iRSCs. (A) Protocol. (B and C) *Grem1-creERT^T; R26-mT/mG* adult induction identifies rare, single cells at the junction of the crypt and villus in the small intestine (green = *Grem1-creERT^T*⁺, red = *Grem1-creERT^T*-negative). Over 1 year, *Grem1*⁺ cells expand to renew the entire periepithelial mesenchymal sheath. By 6 months, they are immediately beneath the intestinal stem cells at the crypt base (B and C). Axes are provided to indicate the longitudinal and circumferential axes (x and y), and “CVA” to designate the crypt-villus, or radial, axis. (D) The mesenchymal expansion was plotted relative to the adjacent epithelial position; 20 well-orientated crypt-villus columns were quantified per mouse. n = 3– 5 mice at each time point; Kruskal-Wallis analysis (p < 0.0001) and post-hoc pairwise Mann-Whitney tests, corrected for multiple comparisons, revealed significant differences (****p <

0.0001, * $p < 0.05$). (E) The sheath was comprised of a reticulated population of stellate cells with long processes that encircled the entire intestinal gland. This cell encapsulated the very base of the intestinal crypt, similar to the position of the cell identified in (C). (F) The *Grem1*⁺ population self-renewed and was multipotent, generating both *Acta2* positive (yellow cell, white arrow) and negative fibroblastic lineages. (G) Transmission electron microscopy: the *Grem1* lineage (yellow arrows) is immediately beneath the epithelial cells. (H) Tissue engineering: representative images from $n = 8$ small-intestinal organoid unit transplants. Small intestines were harvested from 3-week-old, tamoxifen- induced donor mice. In the donor intestines, prior to harvest, there were single *Grem1*⁺ cells (red) near the isthmus of the intestinal gland. After digestion of the donor intestines into organoid units, rare *Grem1*⁺ mesenchymal cells (red) were found within individual organoid units (inset). (I) Four weeks after transplantation, TESIs develop, with the periepithelial mesenchymal sheath recapitulated from the donor *Grem1*⁺ cells (red). E-cadherin staining (green) was used to identify the epithelium.

[0021] **FIG. 7** is a schematic representation showing the OCR stem cell and the perisinusoidal MSC make a complementary contribution to skeletal development, adult homeostasis, and Repair.

[0022] **FIG. 8A-8F** illustrates (A) a candidate panel of genes based on perisinusoidal, niche and important mesenchymal lineage genes. qPCR for each gene was first performed on 100,000 freshly sorted CD45-CD31-Ter119- and then repeated on the same population of cells after 14 days of adherent culture in α MEM plus 10% MSC defined FBS and 1% antibiotics. Represented as fold-change in expression relative to pre-culture levels B, *Grem1* was increased 70x in sorted CD45-CD31-Ter119- cells after 14 days of adherent culture, $n=7$, Wilcoxon rank-sum test, $p=0.016^*$, consistent with a marker of undifferentiated mesenchyme C, Other MSC genes *Nes* and *Cxcl12* were also slightly elevated after culture when compared to their freshly sorted parent population (Mann-Whitney test). All graphs represent mean $\pm$ SD. The increase in post-culture *Grem1* expression was significantly greater than the increase found for *Cxcl12* expression. The increase in *Grem1* expression relative to *Nes* expression after culture did not reach statistical significance. D, *Grem1-creERT* : from 8 potential founders we generated 3 founder lines, confirmed by PCR genotyping for the regions of insertion of the *creERT* into the *Grem1* BAC transgene and also primers specific for *Cre* cDNA. The 5' insertion site PCR was used

subsequently for genotyping (Table 2). These founders were generated on a B6CBA/F2 background, which was then backcrossed 6 generations to C57BL/6J. Only one line (line 3) displayed significant recombination following adult tamoxifen induction. Line 3 was confirmed to be specific for endogenous *Grem1* function by qPCR (E & F) of sorted bone marrow populations based on mesenchymal markers and *Grem1*-recombination, represented as fold change relative to the CD45CD31Ter-119 (+) population. Line 3 was used for all experiments and is available at JAX.

[0023] FIG. 9A-9G illustrates (A) *Grem1-creERT;R26-LSL-ZsGreen;Acta2-RFP* mice (n=3), induced in adulthood revealed that *Grem1* cells were usually *Acta2-RFP* negative. B, When the *Grem1* and *Acta2* positive populations were sorted and cultured, the *Grem1*⁺ population was significantly more clonogenic than the CD45-CD31-Ter-119- *Acta2-RFP* positive cells, p=0.002. In adherent culture the *Grem1*⁺ (green) but *Acta2* negative cells ultimately differentiate into *Acta2* expressing cells (yellow arrows), C, and after sorting these cells and repeating the CFU-F assay, self-renewal was diminished within the *Acta2* expressing descendants of *Grem1*⁺ cells i.e. clonogenicity in first passage of cultured cells was higher in *Grem1*⁺*Acta2*⁻ than those that had become *Grem1*⁺*Acta2*⁺, p=0.003, D. Although, *Grem1* cells traced *Acta2* cells, they never traced *Nes-GFP* expressing cells *in vitro* or *in vivo*. E, Representative flow cytometry of *Grem1-creERT;R26-LSL-TdTomato;Nes-GFP* mice induced in adulthood and then followed for 10 months. Despite *Grem1* OCR stem cells having generated multiple mesenchymal lineages by this time point, none of the *Grem1*-recombined cells had differentiated into *Nes-GFP* cells. Suggesting they are distinct lineages. F & G additional clonal differentiation experiments. In F confirming osteoblast differentiation with alkaline phosphatase detection, and in G, confirming mineralized bone with Alizarin red, and chondrogenesis with Alcian blue.

[0024] FIG. 10A-10L represents (A) color-coded FACS strategy reflected in the color of subsequent graphs, all of which represent mean $\pm$ SD. B, *Grem1*⁺ cells, as a single criterion, significantly enriched for bone marrow CFU-Fs in adherent culture (represented as number of CFU-Fs per 1000 cells for each population, p = 0.002). C, prior to microarray analysis the RNA from FACS cells had to be amplified. We confirmed that amplification was successful and had not distorted the population we repeated *Grem1* qPCR, confirming results from FIG. 8G. *Grem1* recombined cells had higher *Grem1* expression compared to their *Grem1* negative but

mesenchymal (CD45-CD31-Ter-119-) counterparts, in which *Grem1* expression was undetectable. D, Support of microarray findings, *Grem1*-recombination cells had higher expression of *Fap* and *Klf4*. F-H. To determine which signaling pathways (Bmp, Tgfb, Pdgf, Fgf) were active within *Grem1* cells all differentially expressed genes ($\text{fdr} < 0.05$) were assigned to KEGG pathways. These figures show the F, Bmp, and H, Pdgfr/Fgf/Mapk and Vegf I, pathways for which *Grem1*⁺ vs. *Grem1* negative (mesenchyme) was significantly different with $\text{fdr} < .05$. F. The genes in the Bmp pathway (Bmp2, Bmp5, Bmp6, Acvr1, and Id2) are all upregulated (red) so that the pathway is activated. On the other hand, in the pathway also F., although all of the genes (Tgfb1, Dcn, and Ltbp1) are also upregulated, Dcn, and Ltbp1 inhibit Tgfb1, so that there is little, if any net upregulation of the pathway. In the Pdgf/Fgf/Mapk pathway (H), Fgf2, Pdgfa, and Fgr1-3 are all upregulated, but their effect is cancelled all or in part, and perhaps even reversed by the down regulation (blue) of their downstream effectors Sos2, and Mapk1/Erk, so that there is little if any activation of this pathway. There is cross-talk between the Pdgf/Fgf/Mapk and Bmp pathways through the downregulation of Mapk1/Erk, but since Mapk1 inhibits this pathway, its downregulation will either activate or leave the Bmp pathway unaffected. I. The Vegf pathways shows a mix of downregulated (blue) and slightly upregulated genes, that fail to generate a coherent signal constituting pathway activation G. *Grem1-creERT;R26-LSL-ZsGreen* mice (n=4) were induced in adulthood and then examined by flow cytometry for pSmad1,5 positivity, indicative of Bmp signaling. A significantly higher proportion of cells were pSmad1,5⁺ in the *Grem1*⁺ vs. the *Grem1* negative population. J. *Nr2f2* gene expression was on average 4.8 xs higher in the *Grem1*⁺ clones (23 clones) vs. polyclonal *Nes-GFP* derived marrow stromal culture. K. The 23 *Grem1*⁺ derived clones revealed relatively homogeneous expression of the important mesenchymal genes *Runx2*, *Sox9*, *Pparg*, *Myod* and *Sp7*. L. 100% of the 3 distinct human MSC lines we examined expressed *GREM1*.

[0025] FIG. 11A-11J represents A, *Grem1creERT;R26-LSL-TdTomato;2.3colGFP* mice. P1 induction, by 6 weeks in addition to traced osteoblasts (white arrow) and chondrocytes (not shown in this figure) *Grem1*⁺ cells had also traced an extensive network of reticular marrow stromal cells that spanned the endosteal and perivascular areas. These were CD105⁺ (white), yellow arrows, scale bar = 10 μ m. B, 4 weeks (P28) following P1 induction, *Grem1*⁺ cells give rise to approximately 64% of the bone and 50% of the chondrocytes within the metaphysis and epiphysis, C, *Grem1-creERT;R26-LSL-TdTomato* adult induction, traced for 11 months, traced

articular cartilage, here shown on the articular cartilage on the inferior aspect of the femoral head. D, at 12 months, following adult induction the *Grem1* OCR stem cells *Grem1-creERT;R26-LSL-ZsGreen* mice had generated diaphyseal osteoblasts along the endosteal surface (white arrows) and periosteal cells (yellow arrow). 12 months after adult induction *Grem1*⁺ cells, which are green (*Grem1-creERT;R26-LSL-ZsGreen* mice), did not generate adipocytes in the femur E, or in the vertebrae F. Anti-perilipin immunostaining (red) was used to identify adipocytes. G-J, 11 months after adult induction of *Grem1-creERT;R26-LSLTdTomato* single recombined red clones could be harvested from the bone marrow (G), and then expanded and clonally differentiated in bone, I (alizarin red), and cartilage J, (toluidine blue), but again adipocytic differentiation (oil red) was poor H.

[0026] FIG. 12A-12F illustrates (A), *Grem1-creERT; R26-LSL-ZsGreen;R26-LSL-DTA* mice (DTA mice, n=3) vs *Grem1-creERT;R26-LSL-ZsGreen* littermates/related controls (control mice, n=10), tamoxifen induction at P9 (4 doses subcut, 2mg), sacrificed at P23. As one would expect, the DTA mice had a trend towards fewer green cells in the femur, but this did not reach significance, suggesting incomplete ablation. Nevertheless, the DTA mice were significantly lighter than the control mice, B. C, we performed microCT on these mice and there was significantly reduced femur volume in the DTA mice, and a trend towards reduced total bone volume. D, this was associated with reduced trabecular bone development on histology using thresholded measurements and analysis of epiphyseal trabecular bone area in ImageJ (p=0.04). E & F, Induction of *Grem1*⁺ cells at E13.5 results in almost all of the primary spongiosa being labeled with red fluorescence by E21 (*Grem1-creERT;R26-LSL-TdTomato*, n=4).

[0027] FIG. 13A-13E illustrates (A-C), *Grem1-creERT;R26-LSL-TdTomato;2.3colGFP* mice induced in adulthood 1 week before fracture prove that *Grem1*⁺ cells generate both bone and cartilage within the fracture callus (just another example of lineage tracing as shown in main Fig 5, but from different mice). In C, the *Grem1*⁺ OCR-derived osteoblasts are yellow (white arrows) and the OCR stem cell derived chondrocytes are identified by yellow arrows. D, The fracture callus from another *Grem1-creERT;R26-LSL-TdTomato;2.3colGFP* was counterstained by anti-osteocalcin (white) as further proof that the *Grem1*⁺OCR stem cells generate osteoblast (cells that are red (*Grem1*⁺ derived), *2.3ColGFP*⁺ (green) and osteocalcin⁺ (white). Triple + (red, green, white cells) are identified by yellow arrows. The different colors are separated into

distinct images to help with interpretation. E, This figure describes the serial transplantation experiment. *Grem1*⁺ OCR stem cells (red) from one of the fractures described in main FIG. 5, were recovered by callus culture and serially transplanted into a secondary fracture site. Again the secondary fracture showed significant TdTomato fluorescence specifically at the site of engraftment and serial H&E and fluorescence sections reveal the serial engraftment of the red *Grem1* OCR stem cell derived cells into the secondary fracture callus (white arrows), and these cells were positive for Sox9 immunostaining (green) suggesting that they had differentiated into fracture callus chondrocytes (shown in high power, yellow arrows).

[0028] FIG. 14A- 14P illustrates (A) Small intestine of adult *Grem1-creERT;R26-mT/mG* mice 24 hours after tamoxifen induction recombine in single, periepithelial cells at the small intestinal isthmus. B-E the same cells were positive by *Grem1* *in situ* hybridization. F, *Grem1-LacZ* knock in mouse reported *Grem1* expression in the same periepithelial cells at the isthmus (black arrows). In addition, the *Grem1-LacZ* mice also were positive for *LacZ* within the muscularis propria, which was occasionally positive within adult *Grem1-creERT;fluorescent reporter* mice. But, in keeping with our *Grem1-creERT* line, there were never any *Grem1-LacZ* positive cells in the lamina propria of the small intestinal villus. G, *Grem1* recombined cells (Green) divided slowly, *BrdU* incorporation, red, over 1 month of continuous *BrdU* administered via the drinking water. Given the extended *BrdU* administration, all of the epithelial cells have incorporated *BrdU*. H, many of the traced (green) *Grem1*-lineage periepithelial cells expressed *Ng2* a mesenchymal and pericytic marker (also known as *Cspg4*), which was also elevated in our bone marrow microarray. I, *Grem1creERT;R26-LSL-TdTomato* mice induced at P1 and then the jejunum was examined by wholemount confocal microscopy. Patches of glands surrounded by *Grem1*⁺ iRSC derived (red) mesenchymal sheaths can be found. The *Grem1*⁺ iRSC-derived sheath (red) is intimately related to, but distinct from, the overlying s100b⁺ (I) and *Nes*-GFP⁺ (J) glial sheath. The *Grem1*⁺ iRSC-derived sheath is more closely related to the epithelium, than the glial sheath. K, the intimacy between the *Grem1* lineage and the epithelium was best shown by transmission electron microscopy where long cellular processes (red arrows) are very closely opposed to the overlying epithelium, here at the small intestinal crypt base. (L), *Grem1-creERT;R26-mT/mG* mice induced with 6mg of tamoxifen once in adulthood, (6 – 8 weeks) still have fully iRSC-derived (green) small intestinal gland periepithelial mesenchyme (here in the jejunum) at 2 years, confirming self-renewal of the *Grem1* iRSCs. M, *Grem1-creERT;R26-*

Confetti mice, induced at P1. *R26-Confetti* displayed less robust recombination of the reporter than the other reporters we used. Nevertheless, occasional clones of iRSCs could be tracked to confirm that the *Grem1* sheath is derived from one iRSC and that the mesenchymal unit in the small intestine conforms to the epithelial unit, i.e. the periepithelial mesenchymal sheath from one crypt contributes to multiple associated villi, just as in the epithelial compartment. Shown here, with a recombined yellow clone from different sides of the same small intestinal crypt tracked across serial (5µm) sections contributing to the periepithelial sheath in two distinct villi (one villi identified by white, the other by red, arrows). N, The red *Grem1* iRSCs from *Grem1creERT;R26-LSL-TdTomato* mice, can be isolated and cultured to form *in vitro* clones, confirming that *Grem1* iRSCs contribute to small intestinal CFU-Fs. O, expression of *Grem1-creERT* also identified stem/progenitor populations in the hair follicle/skin and the stomach. P, Tissue engineering: Another example of *Grem1*⁺ cells (red) derived from donor *Grem1*⁺ iRSCs within the harvested TESI. Shows that the periepithelial mesenchymal sheath (red) is derived from the donor *Grem1* cells (red), dotted white line outlines the regenerated intestinal epithelium within the TESI.

DESCRIPTION

[0029] In the Summary above and in the Detailed Description below, in the claims below, and in the accompanying drawings, reference is made to particular features (including method steps) of the invention. It is to be understood that the disclosure of the invention in this specification includes all possible combinations of such particular features. For example, where a particular feature is disclosed in the context of a claim, that feature can also be used to the extent possible, in combination with and/or in the context of other particular aspects and embodiments of the invention, and in the invention generally.

1. Introduction

[0030] Regenerative medicine is often described as harnessing the body's regenerative mechanisms in a clinically targeted manner, using the body's capacity to regenerate in ways that are not part of the normal healing mechanism or by artificially amplifying normal mechanisms. Stem cells are pluripotent or multipotent cells with the potential to differentiate into a variety of other cell types, which perform one or more specific functions and have the ability to self-renew. It has been found that stem cells from a variety of sources can be used for multiple therapeutic or prophylactic purposes. For example, mesenchymal stem cells (MSCs) derived from multiple tissues in the adult body are multipotent non-hematopoietic stem cells and are characterized by extensive proliferative ability in an uncommitted state while retaining the potential to give rise to cell types including osteoblasts, myocytes, chondrocytes, adipocytes, endothelial cells and beta pancreatic islet cells. MSCs are present in tissues which arise from the embryonic mesoderm (e.g., hematopoietic cells and connective tissue). Thus, stem cells can be isolated from many tissue sources within the adult body.

[0031] The current understanding in skeletal biology is that a MSC exists in the bone marrow, which is the cellular origin of *all* adult bone, fat and cartilage. MSCs can differentiate into a variety of cell types including cells of connective tissues such as cartilage, muscle, adipose, or tendon. MSCs can be obtained from the bone marrow and can be expanded in vitro.

[0032] Arthritis is a degenerative disease in which cartilage cells lose its function over time, leading to inflammation and other complications accompanied by the loss of cartilage surface on bones, ligaments and joints. Stem cells may be used for orthopedic application including

treatment of treatment of cartilage damage in joints caused by osteoarthritis, aging, and/or mechanical injury.

[0033] Described herein is the discovery that there is an alternate stem cell, the osteochondroreticular stem cell, that is the chief origin of cartilage and bone during development and that traditional mesenchymal stem cells, contribute very little to cartilage. Current approaches for cellular therapy in bone and cartilage regeneration and repair have utilized pooled mesenchymal stem cells. The novel stem cell population described herein provides improved benefits over other stem cells, particularly for the repair of cartilage.

[0034] The new stem cell described herein is called the “osteochondroreticular stem cell” and can be identified and isolated from the bone by expression of the gene Gremlin 1 and/or by particular cell surface markers including, but not limited to, CD200, CD109, CD105. These markers were identified through the process of microarray screens and flow cytometry experiments. These and related markers identified from the screen can be used to isolate human osteochondroreticular stem cells. Described herein is data showing these cells in mice are easily propagated in culture, behave differently to traditional mesenchymal stem cells, are more chondrogenic, and can be easily transplanted into fracture.

[0035] The utility for this discovery is vast for treatment of osteoarthritis. Osteoarthritis describes a degenerative disease whereby the cartilage surrounding tissue at joints wears down, leading to rubbing of bones (Noth U, Steinert A, Tuan R. “Technology Insight: Adult Mesenchymal Stem Cells for Osteoarthritis Therapy.” *Nat Clin Pract Rheumatol*. 2008;4(7):371-380). Symptoms of osteoarthritis include pain and decreased motility in the joint. Current methods of treatment include pain killers to alleviate symptoms and invasive joint replacement surgery for severe cases. Stem cells are cells that can be differentiated into any type of cell depending on the stimulus given. Mesenchymal stem cells have osteogenic (bone) and chondrogenic (cartilage) potential (Solchaga L, Penick K, and Welter J. “Chondrogenic Differentiation of Bone Marrow-Derived Mesenchymal Stem Cells: Tips and Tricks.” *Methods Mol Biol*. 2011;698:253-278). Clinical trials are under way to test the viability of using mesenchymal stem cells to generate cartilage in joints where arthritis is present (Jo C, Lee Y, Shin W, *et al*. “Intra-Articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee: A Proof-of-Concept Clinical Trial.” *Stem Cells*. 2014;32:1254-1266).

[0036] Knee osteoarthritis is a chronic, debilitating condition affecting more than 250 million people world wide (Buchbinder, R. Meniscectomy in Patients with Knee Osteoarthritis and a Meniscal Tear? N Engl J Med 2013; 368:1740-1741). Unfortunately, arthroscopic surgical approaches for this condition are no superior to sham procedure and physical therapy alone (Katz JN, Brophy RH, Chaisson CE, et al. Surgery versus physical therapy for a meniscal tear and osteoarthritis. N Engl J Med 2013;368:1675-1684). Prosthetic joint replacement is the only viable approach for many patients with severe disease. Joint replacement is expensive, complicated and is associated with many specific complications including, infection, joint failure and venous thromboembolism. Despite its limited efficacy, arthroscopy continues to be performed throughout the Western world. Delivery of an effective cellular therapy for osteoarthritis at the time of arthroscopy, would provide enormous benefit to patients and would be of great value.

[0037] There are current clinical trials proceeding looking at the role of allogeneic mesenchymal stem cells for use in knee osteoarthritis (NCT01453738), but pooled MSCs are less chondrogenic than osteochondroreticular stem cells and expansion of the most chondrogenic fraction for delivery, the osteochondroreticular stem cells described herein, has the greatest opportunity for clinical success.

[0038] Accordingly, described herein are methods of identifying and/or isolating osteochondroreticular stem cells. These stem cells are isolated typically from bone tissue and can be distinguished by expression of Gremlin 1 and/or cell surface markers such as CD200, CD109 and CD105, markers identified through microarray screens. Osteochondroreticular stem cells behave differently from mesenchymal stem cells in that they are more chondrogenic.

[0039] Also described herein are therapeutic methods to regenerate the cartilage tissue for treatment of osteoarthritis. It has been determined that OCRs possesses strong chondrogenic potential (able to develop into cartilage) than previously investigated mesenchymal stem cells. The OCRs can be used to developed treatment of osteoarthritis or other diseases where cartilage re-generation would be beneficial.

[0040] Use of these stem cells can regenerate cartilage between bones to alleviate pain and stiffness associated with osteoarthritis. For example, OCR stem cells can be administered directly into joints affected by osteoarthritis. Alternatively, OCR stem cells can be differentiated

into cartilage in culture to aid in study of cartilages or production of cell-based implants for implantation into joints or bone structures.

[0041] In one embodiment isolated OCR stem cells can be expanded, enriched for chondrogenic properties, and used for subsequent injection into joints suffering from osteoarthritis or for application onto or into the bone either to treat or even potentially to prevent fracture in high risk patients.

[0042] In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, to one skilled in the art that the present invention may be practiced without these specific details. In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

2. Definitions

[0043] Unless otherwise defined, all technical and scientific terms used herein are intended to have the same meaning as commonly understood in the art to which this invention pertains and at the time of its filing. Although various methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. However, the skilled should understand that the methods and materials used and described are examples and may not be the only ones suitable for use in the invention. Moreover, it should also be understood that as measurements are subject to inherent variability, any temperature, weight, volume, time interval, pH, salinity, molarity or molality, range, concentration and any other measurements, quantities or numerical expressions given herein are intended to be approximate and not exact or critical figures unless expressly stated to the contrary. Hence, where appropriate to the invention and as understood by those of skill in the art, it is proper to describe the various aspects of the invention using approximate or relative terms and terms of degree commonly employed in patent applications, such as: so dimensioned, about, approximately, substantially, essentially, consisting essentially of, comprising, and effective amount.

[0044] Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein, and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lan, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Principles of Neural Science, 4th ed., Eric R. Kandel, James H. Schwartz, Thomas M. Jessell editors. McGraw-Hill/Appleton & Lange: New York, N. Y. (2000). Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0045] The term "acceptable carrier" as used herein, means excipients, emollients, and stabilizers or stabilizing agents or other acceptable materials, compositions, or structures involved in holding, carrying, transporting, or delivering any subject cell or composition. Each means must be "acceptable" in the sense of being compatible with the other ingredients of a subject composition and not injurious to the subject.

[0046] The term "administering" as used herein, means delivery, for example of an OCR stem cell to a subject.

[0047] The term "bone tissue" as used herein is tissue that includes bone or bone marrow.

[0048] The terms "express," "expression," and "expressing," as used herein with respect to gene products, indicate that the gene product of interest is produced by the cell at a detectable level. "Significant expression" refers to expression of the gene product of interest to 10% above the minimum detectable expression. Cells with "high expression" or "high levels" of expression of a given expression product are the 10% of cells in a given sample or population of cells that exhibit the highest expression of the expression product. Cells with "low expression" of a given

expression product are the 10% of cells in a given sample or population of cells that exhibit the lowest expression of the expression product (which can be no expression).

[0049] The terms "isolated," "isolating," "purified," "purifying," "enriched," and "enriching," as used herein with respect to cells, means that the OCR stem cells at some point in time were separated, sorted and capable of directed differentiation. "Highly purified," "highly enriched," and "highly isolated," when used with respect to cells, indicates that the cells of interest are at least about 70%, about 75%, about 80%, about 85% about 90% or more of the cells, about 95%, at least 99% pure, at least 99.5% pure, or at least 99.9% pure or more of the cells, and can preferably be about 95% or more of the differentiated cells.

[0050] The term "multipotent" as used herein, refers to a property of any stem cell or progenitor cell, meaning that it has the ability to differentiate into two or more different cell types. Pluripotent stem cells, such as embryonic stem cells, can give rise to all of cell types, thus multipotent cells are less potent than pluripotent cells. Adult stem cells are considered multipotent.

[0051] The term "osteochondroreticular stem cell" or "OCR stem cell" as used herein, refers to lineage-specific Grem1+ skeletal stem cells. OCR stem cells typically reside within the bone or bone marrow.

[0052] The term "population" as used herein when used with respect to cells, means a group or collection of cells that share one or more characteristics. The term "subpopulation," when used with respect to cells, refers to a population of cells that are only a portion or "subset" of a population of cells.

[0053] The term "progenitor cell" is a cell that, like a stem cell, has a tendency to differentiate into a specific type of cell, but is already more specific than a stem cell and is pushed to differentiate into its "target" cell. In certain embodiments, the OCR stem cell is a lineage-specific progenitor cell that is pushed to differentiate into osteoblasts, chondrocytes, and reticular marrow stem cells, but not adipocytes.

[0054] The term “skeletal cell sample” as used herein means, a cell sample obtained from bone tissue. Skeletal cell samples include multiple cell types including OCRs. OCRs are isolated utilizing the techniques herein such as sorting based on Grem1 expression.

[0055] The term “stem cells” (or blank cells) are undifferentiated cells that can divide or differentiate into specialized cells, replacing dying cells or damaged tissues. There are two broad types of stem cells: embryonic stem cells (ESCs) and adult stem cells (somatic stem cells).

[0056] The terms "subject," "host," and "patient," as used herein, are used interchangeably and mean a mammalian animal being treated with the present compositions, including, but not limited to, vertebrates, simians, humans, felines, canines, equines, rodents (including rats, mice and the like), bovines, porcines, ovines, caprines, mammalian farm animals, mammalian sport animals, and mammalian pets.

[0057] The terms "substantially pure," “substantially purified,” and “substantially enriched” as used herein with respect to cells means the isolated cell population of cells that includes at least 80% pure, and preferably at least 85% pure, at least 90% pure, at least 95% pure, at least 97% pure, at least 98% pure, at least 99% pure, at least 99.5% pure, or at least 99.9% pure cells of the type in question, for example, Grem1⁺ OCR stem cells. Percentage purity refers to the percentage of the cell type in question relative to all cells in the sample.

[0058] As used herein, a “therapeutic agent” means a compound or molecule capable of producing an effect. Preferably, the effect is beneficial.

[0059] As used herein, "therapeutically effective amount" means an amount sufficient to treat a subject.

[0060] As used herein, the terms "treatment," "treating," and “treat” and the like, as used herein refer to obtaining a desired medical effect. The effect may be prophylactic in terms of completely or partially preventing a condition (i.e., disease, degeneration, disorder or injury) or symptom thereof and/or may be therapeutic in terms of a partial or complete cure or repair of the condition and/or adverse effect attributable to the same. "Treatment," includes any treatment of a condition in a mammal, particularly in a human, and includes: (a) preventing the condition or disease or symptom thereof from occurring in a subject which may be predisposed to the condition or

disease but has not yet been diagnosed as having it; (b) inhibiting the condition or symptom thereof, such as, arresting its development; and (c) relieving, alleviating or ameliorating the condition or symptom thereof, such as, for example, causing regression of the condition or symptom thereof.

3. Overview

[0061] The newly-identified OCR stem cells are integrally involved in maintenance and repair of the postnatal skeleton. One model that has been previously suggested is that perisinusoidal mesenchymal stem cells (MSCs) give rise to osteoblasts, chondrocytes and marrow stromal cells, as well as adipocytes. However, the existence of such an endogenous MSC has not been proven through fate-mapping experiments. The discovered OCR stem cells that express the BMP antagonist gremlin 1 (*Grem1*) are found in bone and bone marrow. OCR stem cells self-renew and generate osteoblasts, chondrocytes and reticular marrow stromal cells, but not adipocytes. In adulthood, OCR stem cells are concentrated within the metaphysis of long bones and are distinct from traditional perisinusoidal, nestin-expressing MSCs. OCR stem cells are important for bone development, adult skeletal homeostasis and fracture repair, while nestin⁺ MSCs contribute little to skeletogenesis. Incidentally, it has been discovered that *Grem1* expression also identifies intestinal reticular stem cells (iRSCs) that can be transplanted and are the cell of origin for the periepithelial intestinal mesenchymal sheath.

[0062] Furthermore, OCR stem cells, when transplanted to a fracture site, contribute to bone repair. It is therefore possible that drugs or other therapies can be developed to stimulate the production of OCR stem cells and improve the body's ability to repair bone injury—a process that declines significantly in old age. These cells are particularly active during development, but they also increase in number in adulthood after bone injury. The study also showed that the adult OCR stem cells are distinct from MSCs, which play a role in bone generation during development and adulthood. Researchers presumed that MSCs were the origin of all bone, cartilage, and fat, but recent studies have shown that these cells do not generate young bone and cartilage. Without being bound by theory, OCR stem cells actually fill this function and that both OCR stems cells and MSCs contribute to bone maintenance and repair in adults.

[0063] Described herein are methods of identifying and/or isolating OCR stem cells. These stem cells are isolated typically from bone tissue and can be distinguished by expression of Gremlin 1 and/or cell surface markers such as CD200, CD109 and CD105, markers identified through microarray screens. Osteochondroreticular stem cells behave differently from mesenchymal stem cells in that they are more clonogenic and chondrogenic.

[0064] Also described herein are therapeutic methods to regenerate the cartilage tissue for treatment of osteoarthritis. It has been determined that OCRs possesses strong chondrogenic potential (able to develop into cartilage) than previously investigated mesenchymal stem cells. The OCRs can be used to developed treatment of osteoarthritis or other diseases where cartilage re-generation would be beneficial.

[0065] Use of these stem cells can re-generate cartilage between bones to alleviate pain and stiffness associated with osteoarthritis. For example, OCR stem cells can be administered directly into joints affected by osteoarthritis. Alternatively, OCR stem cells can be differentiated into cartilage in culture to aid in study of cartilages or production of cell-based implants for implantation into joints or bone structures. Further, the method by which OCRs were identified (through microarray screens) can be used to identify other types of stem cells that have chondrogenic potential.

[0066] In one embodiment, isolated OCR stem cells can be expanded, enriched for chondrogenic properties, and used for subsequent injection into joints suffering from osteoarthritis or for application onto or into the bone either to treat or even potentially to prevent fracture in high risk patients.

[0067] A specific method embodiment disclosed herein involves isolating OCR stem cells that promote regeneration of cartilage tissue useful for treatment of diseases of the bone and cartilage involving the steps of extracting bone and/or bone marrow from a subject, identifying the OCR stem cells by expression of the gene Gremlin 1 (Grem1) and/or by particular cell surface markers and isolating OCR stem cells from the bone and/or bone marrow. .

[0068] In the identification step, the OCR stem cells are identified by expression of the gene Gremlin 1 (Grem1) and/or by particular cell surface markers CD200, CD109, and CD105. These markers can be identified through the process of microarray screens and flow cytometry

experiments as described herein in Example 2 and FIG. 2. These and related markers identified from the screen can be used to isolate the OCR stem cells to promote regeneration of cartilage or bone. An inducible *Cre* line, driven by the enhancer elements of the BMP antagonist *Grem1*, was generated to identify and trace rare skeletal stem cells *in vivo*. Adult *Grem1* osteochondroreticular (OCR) stem cells were found beside the growth plate and the trabecular bone, where they generated and maintained articular and growth plate cartilage, bone and reticular marrow stromal cells, but not fat.

4. Detailed Description of the Embodiments

[0069] The discovery of a new Grem-1+ cell type, herein referred to as OCR stem cells, which is programmed to produce certain cell types, namely bone and cartilage cells, leads to a number of therapeutic possibilities. Accordingly, methods of isolating OCR stem cells, compositions and kits comprising them, are provided. Methods for treating diseases relating to damaged cartilage or bone are also provided, such as administration via direct injection into a joint to help repair cartilage, injection into subchondral defects, bone fractures, and the engineering various cell-based scaffolds for implantation for bone or cartilage repair, or bone paste materials. See, for example, U.S. Patent Pub. No. 20140147419.

A. Methods of Isolating OCR Stem Cells for Generation of Bone and Cartilage

[0070] Certain embodiments described herein relate to for isolating OCR stem cells that promote regeneration of cartilage tissue useful for treatment of diseases of the bone and cartilage resulting from age, gender, genes, excess weight, poor diet, sedentary lifestyle, injury or trauma (leading to degenerative arthritis), abnormal metabolism (such as gout and pseudogout), inheritance (such as in osteoarthritis), infections (such as in the arthritis of Lyme disease), and an overactive immune system (such as rheumatoid arthritis and systemic lupus erythematosus). The lineage restricted progenitor cells, or OCR stem cells express CD105, a well-established marker of bone marrow, (Grem1+ CD105 OCR stem cells) and have skeletal tissue fates. Therefore, in certain embodiments, the method comprises the steps of: (a) identifying a subject in need of cartilage or bone repair; (b) extracting bone and/or bone marrow from a subject; (c) identifying the OCR stem cells by expression of the gene Gremlin 1 (Grem1) and/or by particular cell surface markers selected from the group consisting of CD200, CD109, and CD105; and (c)

isolating OCR stem cells from the bone and/or bone marrow via enzymatic digestion wherein the isolated OCR stem cells promote regeneration of cartilage tissue and/or bone.

[0071] In some embodiments, the isolated Grem1+ CD105 OCR stem cells are subjected to conditions that promote differentiation into osteoblasts, chondrocytes, and reticular marrow stromal cells that are useful for regeneration of cartilage tissue for treatment of diseases described herein. The conditions that promote differentiation comprise culturing the OCR stem cells in the presence of medium that comprises bone morphogenic protein (BMP). Other embodiments include using the immunophenotype of OCR stem cells or the presence of Grem1 expression to isolate these cells.

Extraction and Isolation of OCR Stem Cells

[0072] OCR stem cells are obtainable from bone marrow by minimally invasive techniques and can be expanded in culture and permitted to differentiate into the desired lineage. OCR stem cells can be isolated based either on surface markers ('prospectively') or by establishing clonal adherent cultures. As long as clonogenicity assays remain the mainstay of characterization of cells isolated based on surface markers, and as long as cell culture remains necessary prior to transplantation in vivo, isolation by either surface marker or by adherence and clonogenicity yield essentially identical results.

[0073] In a select embodiment, the method comprises preparing a cell suspension from bone marrow. Such a cell suspension generally comprises OCRs and is separated from the cell suspension using any convenient method known in the art, for example, a fluorescence-based sorting techniques and expression labels. Suitable labels include, but are not limited to green fluorescent protein (GFP), varieties of other fluorescent proteins including yellow and red, other optical labels utilized for cell separation whose expression is driven by a Grem promoter, Grem1, or other cell surface markers whose expression is highly correlated with the expression of GFP or its derivatives, or Grem1, or both. Anti-Grem1 antibody is preferred.

[0074] Techniques for labeling, sorting, fluorescence activated cell sorting (FACS), and enrichment of cells are well known in the art. Useful examples are described in WO 2001/022507 and U.S. application Ser. No. 13/391,251 (US 2012-0220030 A1), which are hereby incorporated by reference in their entirety, and specifically for their description of cell

labeling, sorting, and enrichment. The cells can be identified, separated, and/or enriched based on cell markers. It will be understood by those of skill in the art that the stated expression levels reflect detectable amounts of the marker protein on the cell surface. Generally, cell markers can be assessed by staining or labeling cells with probes that specifically bind the marker of interest and that generate a detectable signal.

Differentiation

[0075] OCR stem cells can be cultured by a variety of means known to the art. For example, OCR stem cells can be plated (e.g., about 100,000 cells per well) for 2D culture. As another example, OCR stem cells can be centrifuged (e.g., about 2 million cells) to form a 3D pellet. Monolayer (2D) or 3D cell pellets can be cultured in a suitable growth medium. Methods of culturing OCR stem cells are generally known in the art and such methods can be adapted so as to provide optimal conditions for differentiation. OCR stem cells can be induced to differentiate in a first medium (e.g., a medium with serum and missing BMP) and then expanded in a second medium (e.g., a medium with BMP).

[0076] OCR stem cells can be expanded on an expansion medium. An expansion medium would usually be simply the base media (alphaMEM plus 10% fetal calf serum), that could include additives depending on the desired differentiation for the expanded cells. If just expanding the cells, the “base media” would be sufficient.

[0077] Differentiation can be induced by the application of specific growth factors. The transforming growth factor beta (TGF-beta) superfamily member proteins such as the bone morphogenetic proteins (BMPs) are important factors of chondrogenic and osteogenic differentiation.

[0078] Differentiation of OCR stem cells to the osteogenic lineage may be achieved by culture in osteogenic medium. For example, OCR stem cells are seeded at 3,000/cm² in maintenance medium (DMEM, 1 g/l glucose, 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin and 50 U/ml streptomycin) in 6-well, 12-well and chamber slides for 24 h before changing to osteogenic media (maintenance medium, 10 nM dexamethasone, 25 µg/ml ascorbic acid and 10 mM β-glycerophosphate). Cells are then maintained for up to 28 days with a media change every 3-4 days. After 14 days cells in the chamber slides may be fixed in 4% PFA and stored at 4°C in PBS

for immunohistochemistry. After 14 and 28 days the cells are stained with alizarin red S for calcium, and von Kossa for calcium phosphate. RNA may also be extracted for analysis using the Nucleospin RNA extraction kit according to the manufacturer's instructions (Macherey Nagel) and protein samples may be extracted for analysis.

[0079] Differentiation of OCR stem cells to the chondrogenic lineage in certain embodiments may be achieved by culture in chondrogenic medium. For example, OCR stem cells are counted and resuspended at 5×10^5 cells/ml in chondrogenic media (DMEM with Cambrex chondrogenic single aliquots) with or without 10 ng/ml TGF. β 1 (Cambrex) and then 500 μ l aliquots were put into 15 ml tubes before centrifugation at 150.g at room temperature for 10 min and incubated at 37°C for 2 days. After two days the tubes will contain loose round pellets. Pellets are maintained for 21 days with a media change every 3-4 days before RNA is isolated using Trizol (Invitrogen) or cell pellets are fixed in 4% PFA and embedded for cryosectioning. Serial sections are made before slides are stored at -80 degrees Celsius for immunohistochemistry.

[0080] Any suitable method of culturing ORC stem cells may be used, and any suitable container may be used to propagate ORC stem cells. Suitable containers include those described in US Patent Publication US2007/0264713 (Terstegge). Containers may include bioreactors and spinners, for example. A "bioreactor" is a container suitable for the cultivation of eukaryotic cells, for example animal cells or mammalian cells, such as in a large scale. A typical cultivation volume of a regulated bioreactor is between 20 ml and 500 ml.

[0081] Bioreactors may comprise a regulated bioreactor, in which one or more conditions may be controlled or monitored, for example, oxygen partial pressure. Devices for measuring and regulating these conditions are known in the art. For example, oxygen electrodes may be used for oxygen partial pressure. The oxygen partial pressure can be regulated via the amount and the composition of the selected gas mixture (e.g., air or a mixture of air and/or oxygen and/or nitrogen and/or carbon dioxide). Suitable devices for measuring and regulating the oxygen partial pressure are described by Bailey, J. E. (Bailey, J. E., Biochemical Engineering Fundamentals, second edition, McGraw-Hill, Inc. ISBN 0-07-003212-2 Higher Education, (1986)) or Jackson A. T. Jackson A. T., Verfahrenstechnik in der Biotechnologie, Springer, ISBN 3540561900 (1993)).

[0082] Other suitable containers include spinners. Spinners are regulated or unregulated bioreactors, which can be agitated using various agitator mechanisms, such as glass ball agitators, impeller agitators, and other suitable agitators. The cultivation volume of a spinner is typically between 20 ml and 500 ml. Roller bottles are round cell culture flasks made of plastic or glass having a culture area of between 400 and 2000 cm². The cells are cultivated along the entire inner surface of these flasks; the cells are coated with culture medium accomplished by a "rolling" motion, i.e. rotating the bottles about their own individual axis.

[0083] Alternatively, culture may be static, i.e. where active agitation of the culture/culture media is not employed. By reducing agitation of the culture, aggregates of cells may be allowed to form. While some agitation may be employed to encourage distribution and flow of the culture media over the cultured cells this may be applied so as not to substantially disrupt aggregate formation. For example, a low rpm agitation, e.g. less than 30 rpm or less than 20 rpm, may be employed. In preferred embodiments, cloning cylinders are used.

Expansion of OCR stem cells

[0084] Expansion of OCR stem cells refers to the increase in population of OCR stem cells in a culture, achieved through cell division. In some embodiments, OCR stem cells are obtained by culture of bone marrow stromal cells alone or in the presence of BMP for sufficient time to expand a single MSC to a population of more than 1×10^3 stem cells. The culture may initially contain more than one OC stem cell. The culture time to expand the OCR stem cells may be between 5 and 50 days, more preferably between 10 and 45 days and more preferably less than one of 45 days, 40 days, 35 days, 30 days, 25 days, 20 days or 15 days.

[0085] In some embodiments, cultures may also comprise other cells, e.g. non-stem cells associated with the stem cells in the tissue from which the stem cells are collected, and/or supporting cells, e.g. feeder cells. Cells used to initiate a culture of stem cells will preferably contain a high proportion of the respective stem cells, e.g. at least 60% stem cells, more preferably one of at least 70% stem cells, 80% stem cells, 90% stem cells, 95% stem cells, 96% stem cells, 97% stem cells, 98% stem cells, 99% stem cells or 100% stem cells. Cells, e.g. cells collected from previous cell culture or from live animals or humans, may be enriched prior to initiating cell culture, e.g. by enriching for markers such as Grem1 CD200, CD109, and CD105

Marker enrichment may be performed by cell sorting, e.g. FACS. In a specific embodiment, OCR cells are sorted using the Smartflare system (Millipore) based on Grem1 expression (www.emdmillipore.com/US/en/life-science-research/genomic-analysis/SmartFlare-Live-Cell-RNA-Detection/ZdGb.qB.KCcAAAFLAQs0i.s1.nav).

[0086] OCR stem cells described herein may be cells from any type of animal. Preferably they are mammalian. In some embodiments they are human. In other embodiments they are from a non-human mammal. The non-human mammal may be a domestic pet, or animal kept for commercial purposes, e.g. a race horse, or farming livestock such as pigs, sheep or cattle. Non-human mammals include rabbits, guinea pigs, rats, mice or other rodents (including any animal in the order Rodentia), cats, dogs, pigs, sheep, goats, cattle (including cows, e.g. dairy cows, or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primates.

[0087] The culture methodology described above is preferably performed in vitro. The term "in vitro" is intended to encompass experiments with cells in culture whereas the term "in vivo" is intended to encompass experiments with intact multi-cellular organisms.

[0088] In certain embodiments, culture of cells in the presence of a factor, such as BMP refers to culture of cells under conditions in which the cells being cultured are able to come into contact with the factor. In preferred embodiments this comprises culturing cells in culture media containing the factor. The culture media may be fluid, e.g. liquid or gel, and may contain MBP in addition to the normal nutrients, growth factors and matrix material. The factor will preferably be present in non-trace amounts. For example, the concentration of the factor in the culture media may range between about 1.0 ng/ml culture media to about 1000 ng/ml culture media. More preferably, the concentration of the factor in the culture media may be between about 5 ng/ml culture media and 200 ng/ml culture media, or between about 20 ng/ml culture media and 170 ng/ml culture media.

[0089] As mentioned above, cell culture media may include growth factors, cytokines, hormones, and various nutrients. Illustrative growth factors may include transforming growth factor-beta (TGF- β), fibroblast growth factors (FGFs), insulin like growth factors (IGFs), bone morphogenic proteins (BMPs); illustrative cytokines may include cytokine-like 1 (Cyt11);

illustrative hormones may include human growth hormone (HGH); and testosterone; and illustrative nutrients may include ascorbic acid, pyruvate, hyaluronic acid and amino acids.

[0090] The properties of cells obtained from culture in the presence of a factor, such as BMP, may be compared against cells of the same type obtained from culture in control conditions. "Control conditions" or "control culture" refers to culture of the cells under conditions in which the cells being cultured do not come into contact with the factor. As such, control conditions may comprise culture in culture media that contains the normal nutrients, growth factors and matrix material but no factor. Examples of control culture media for culture of OCR stem cells include serum free media such as that Brunner, D., et al., "Serum-free cell culture: the serum-free media interactive online database," ALTEX 27(1), 53-62, 2010. Other culture conditions known in the art are disclosed in Panagiota, A., et al., "Characterization of the Optimal Culture Conditions for Clinical Scale Production of Human Mesenchymal Stem Cells", Stem Cells, 2005.

[0091] Exemplary maintenance media for cell culture in certain embodiments may comprise DMEM, 1,000 mg/l glucose supplemented with 10% fetal bovine serum (FBS) with 0.1% penicillin/streptomycin and 2 mM L-glutamine at 37° C in a humidified 5% CO₂ incubator. Media may be changed at three-day intervals and the cells subcultured every 4-5 days (about 80% confluency).

B. Isolated OCR stem cells

[0092] Isolated Grem1+, and optionally CD105+, OCR stem cells, are made according to the methods described above, in this paragraph and throughout the specification. Preferably, the isolated Grem1+, and optionally CD105+, OCR stem cells in embodiments of the invention are at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, at least 97% pure, at least 98% pure, at least 99% pure, at least 99.5% pure, or at least 99.9% pure Grem1+, and optionally CD105+, OCR stem cells.

[0093] In addition, certain embodiments of the invention comprise a composition comprising an acceptable carrier and the isolated Grem1+, and optionally CD105+, stem cells described above in this paragraph and described throughout the specification. Certain embodiments of the invention also include substantially pure isolated Grem1+ OCR stem cells from bone and bone marrow, which express the cell marker Grem1+ and optionally CD105+. In other embodiments, methods are provided for treating osteoarthritis and bone fracture by administering a

therapeutically effective amount of the composition. The composition may be administered to tissue surround the fracture,

C. Therapeutic Methods

[0094] The OCR stem cells identified herein can be used for treating disease, degeneration or injury of bone and/or cartilage tissue. Examples of conditions that may be treated include, but are not limited to, arthritis; osteoarthritis; osteoporosis; osteochondrosis; osteochondritis; osteogenesis imperfecta; osteomyelitis; osteophytes; achondroplasia; costochondritis; chondroma; chondrosarcoma; herniated disk; Klippel-Feil syndrome; osteitis deformans; osteitis fibrosa cystica, a congenital defect that results in absence of a tissue; accidental tissue defect or damage; fracture; wound; joint trauma; an autoimmune disorder; diabetes; Charcot foot; tissue resection; periodontal disease; implant extraction; or tumor resection. The OCR stem cells are particularly suitable for treating conditions such as osteoarthritis, osteoporosis and fracture.

[0095] In certain embodiments, *Grem1* OCR stem cells are harvested from a donor animal, expanded *in vitro*, and transplanted, directly and serially, into a site of need in a recipient animal. A site of need includes, but is not limited to, the space of a joint, cartilage tissue of the joint, the articular tissue of the bones forming the joint, locations on a bone outside of a joint; a site of fracture or defect and tissue surrounding a fracture or defect.

[0096] The discovery of analogous is likely to be important in intestinal replacement and repair and may inform mesenchymal hierarchy in other complicated connective tissues including the tumor microenvironment. iRSCs will be used in settings requiring the support of epithelium, such as in generation of new tissue for intestinal failure (such as short gut, inflammatory ulceration, peptic ulceration, fistulae), it could also be used for screening therapeutic targets in cancer, when the epithelial-mesenchymal partnership is more predictive of drug sensitivity than epithelial-specific tissue alone and finally could be used to help mature epithelium to better model intestinal microbiome interactions.

[0097] Accordingly, in a further embodiment, disclosed is a method that involves obtaining a population of isolated intestinal reticular stem cells (iRSCs); and administering the population of iRSCs into an intestine of a subject in need thereof. Upon administration, the population of

iRSCs is able to generate periepithelial mesenchymal sheath in the intestine of the recipient. Typically, the isolated iRSCs are isolated based on expression of *Grem1*. The isolated *Grem1* expressing intestinal cells may be subjected to cell culture conditions to generate a clone and further a gut organoid suitable for implantation.

[0098] A further method embodiment involves (a) obtaining an intestinal cell sample from a subject, wherein the intestinal cell sample comprises intestinal reticular stem cells (iRSCs); and (b) isolating from the cell sample a population of cells that express Gremlin 1 (*Grem1*), to produce a sample of isolated iRSCs.

Treatment of Diseases Associated with Bone or Tissue Damage.

[0099] In certain embodiments, OCR stem cells are administered to treat diseases of the bone or cartilage such as osteoarthritis. As an example, a subject in need may have damage to a tissue, such as bone tissue, and the method provides an increase in biological function of the tissue by at least 5%, 10%, 25%, 50%, 75%, 90%, 100%, or 200%, or even by as much as 300%, 400%, or 500%. As yet another example, the subject in need may have a disease, disorder, or condition, and the method provides an administration of OCR stem cells or compositions comprising them, sufficient to ameliorate or stabilize the disease, disorder, or condition. For example, the subject may have a disease, disorder, or condition that results in the loss, atrophy, dysfunction, or death of bone and/or cartilage cells. Exemplary treated conditions include arthritis; osteoarthritis; osteoporosis; osteochondrosis; osteochondritis; osteogenesis imperfecta; osteomyelitis; osteophytes (i.e., bone spurs); achondroplasia; costochondritis; chondroma; chondrosarcoma; herniated disk; Klippel-Feil syndrome; osteitis deformans; osteitis fibrosa cystica, a congenital defect that results in the absence of a tissue; accidental tissue defect or damage such as fracture, wound, or joint trauma; an autoimmune disorder; diabetes (e.g., Charcot foot); cancer; a disease, disorder, or condition that requires the removal of a tissue (e.g., tumor resection); periodontal disease; and implant extraction. In a further example, the subject in need may have an increased risk of developing a disease, disorder, or condition that is delayed or prevented by the method.

[0100] The methods, compositions, and devices of the application can include concurrent or sequential treatment with one or more of enzymes, ions, growth factors, and biologic agents, such as thrombin and calcium, or combinations thereof. The methods, compositions, and devices

of the application can include concurrent or sequential treatment with non-biologic or biologic drugs.

Bone Fracture

[0101] In certain embodiments, OCR stem cells are administered to treat bone fracture. OCR stem cells stimulate bone regeneration following injury and contribute to improved wound healing in bone. OCR stem cells provide improvements in the speed of bone fracture repair enabling a reduction in the recovery time from injury. Administration of OCRs is preferably to the tissue surrounding the fracture. This may include administration directly to bone tissue in which the fracture has occurred. Administration may be to connective tissue surrounding the bone or fracture or to vasculature (e.g. blood vessels) near to and supplying the bone. Administration may be directly to the site of injury and may be to a callus formed by initial healing of the wound.

[0102] In certain embodiments, fractures include closed or open and simple or multi-fragmentary fractures. In closed fractures the skin remains intact, whilst in an open fracture the bone may be exposed through the wound site, which brings a higher risk of infection. Simple fractures occur along a single line, tending to divide the bone in two. Multi-fragmentary fractures spilt the bone into multiple pieces. Other fracture types include, compression fracture, compacted fracture, spiral fracture, complete and incomplete fractures, transverse, linear and oblique fractures and comminuted fractures. In most subjects bone healing (fracture union) occurs naturally and is initiated following injury. Bleeding normally leads to clotting and attraction of white blood cells and fibroblasts, followed by production of collagen fibers. This is followed by bone matrix (calcium hydroxyapatite) deposition (mineralization) transforming the collagen matrix into bone. Immature re-generated bone is typically weaker than mature bone and over time the immature bone undergoes a process of remodeling to produce mature "lamellar" bone. The complete bone healing process takes considerable time, typically many months.

[0103] Bones in which fractures occur and which may benefit from treatment using OCRs include all bone types, particularly all mammalian bones including, but not limited to, long bones (e.g. femur, humerus, phalanges), short bones (e.g. carpals, tarsals), flat bones (e.g. cranium, ribs, scapula, sternum, pelvic girdle), irregular bones (e.g. vertebrae), sesamoid bones (e.g. patella).

Bone fracture also includes pathological porosity, such as that exhibited by subjects with osteoporosis.

D. Compositions and Kits

[0104] For the administration in the prevention and/or treatment of a bone or cartilage disorder, such as joint disorders (e.g., osteoarthritis), ORC stem cells of the invention can be formulated in a suitable composition, comprising ORC stem cells of the invention, in a therapeutically or prophylactically effective amount, together with a suitable pharmaceutically acceptable vehicle. The composition of the invention can be formulated according to the chosen form of administration. For example, a composition is prepared in a liquid dosage form, e.g., as a suspension, to be injected into the subject in need of treatment. The composition of the invention can contain a prophylactically or therapeutically effective amount of the cells of the invention, preferably in a substantially purified form, together with the suitable vehicle in the appropriate amount in order to provide the form for proper administration to the subject. Suitable carriers or components typically used alone, or in combination, are known in the art and include, but are not limited to, water, saline, dextrose, and glycerol.

[0105] The compositions of the invention, if desired, can also contain, when necessary, additives to enhance, control, or otherwise direct the intended therapeutic effect of the cells comprising said pharmaceutical composition, and/or auxiliary substances or pharmaceutically acceptable substances, such as minor amounts of pH buffering agents, tensioactives, co-solvents, preservatives, etc. For example, the pharmaceutical composition preferably comprises constituents which protect, culture, and maintain the stem cells for a desired treatment period of 5 to 14 days or more thereby extending the release of therapeutic extracellular factors from the encapsulated cells. The pharmaceutical composition can also contain constituents to maintain the stem cells in undifferentiated form. The stability of the cells in the pharmaceutical composition of the invention can be improved by means of adding additional substances, such as, for example, amino acids such as aspartic acid, glutamic acid, etc. Pharmaceutically acceptable substances that can be used in the pharmaceutical composition of the invention are known, in general, by the skilled person in the art and are normally used in the manufacture of cellular compositions.

[0106] The compositions may also include auxiliary substances such as growth factors, cytokines, hormones, and various nutrients. Illustrative growth factors may include transforming growth factor-beta (TGF- β), fibroblast growth factors (FGFs), insulin like growth factors (IGFs), bone morphogenic proteins (BMPs); illustrative cytokines may include cytokine-like 1 (Cyt11); illustrative hormones may include human growth hormone (HGH); and testosterone; and illustrative nutrients may include ascorbic acid, pyruvate, hyaluronic acid and amino acids.

[0107] The compositions may also include additional therapeutic agents routinely used in the art for alleviation of pain and inflammation and include, but are not limited to, narcotics, corticosteroids, anti-inflammatories including ibuprofen, naproxen, diclofenac, anti-biotics, analgesics, and natural remedies,

[0108] In one example of a therapeutic composition, OCR stem cells are produced by any of the methods described herein. OCR stem cells are then prepared for application to subjects in need of the cells. OCR stem cells can also be prepared in pharmaceutical dosages (e.g., in a pharmaceutically acceptable solution) and stored in appropriate containers. The OCR stem cells can be stored in an appropriate manner (e.g., frozen) until needed. Additionally, the pharmaceutical dosages can be placed in pre-prepared syringes, catheters or other medical devices appropriate for delivery to an affected joint. One of skill in the art will recognize that dosage amount, needle length and other such parameters can be adjusted for any individual preparation.

[0109] A pharmaceutical composition containing OCR stem cells of the present invention may be stored until use by means of conventional methods known by the skilled person in the art. For short term storage (less than 6 hours) the pharmaceutical composition containing said cells may be stored at or below room temperature in a sealed container with or without supplementation with a nutrient solution. Medium term storage (less than 48 hours) is preferably performed at 2-8°C, the pharmaceutical composition comprising an iso-osmotic, buffered solution in a container composed of or coated with a material that prevents cell adhesion. Longer term storage is preferably performed by appropriate cryopreservation and storage under conditions that promote retention of cellular function.

[0110] OCR stem cells produced, stored, or banked may be administered to non-autologous recipients in either prepared dosages or pre-dosage containers and can be shipped to medical facilities through any approved delivery system (governmentally approved and/or commercial). OCR stem cells can be delivered directly from the manufacturer or via an intermediary.

E. Administration Of Therapeutic Compositions

[0111] The administration of the pharmaceutical composition of the invention to the subject in need thereof can be carried out by conventional means. In a particular embodiment, said pharmaceutical composition can be administered to the subject in need by administration using devices such as syringes, catheters, trocars, cannulae, etc. for direct injection into a joint to help repair cartilage, injection into subchondral defects, bone fractures, etc. engineering various cell-based scaffolds for implantation for bone or cartilage repair, or bone paste materials. In any case, the pharmaceutical composition of the invention will be administered using the appropriate equipment, apparatus, and devices which are known by the skilled person in art in a therapeutically or prophylactically effective amount, together with a suitable pharmaceutically acceptable vehicle.

[0112] OCR stem cells disclosed herein can be applied by several routes including direct injection into the affected anatomical site. A pharmaceutical composition containing the cells may be injected in a single bolus, through a slow infusion, or through a staggered series of applications separated by several hours, several days or weeks. In any case, the pharmaceutical composition of the invention will be administered to the target tissue using the appropriate equipment, apparatus, and devices which are known by the skilled person in art in a therapeutically or prophylactically effective amount.

[0113] One of skill in the art will recognize that cell numbers (e.g., dosage amount) will vary depending upon multiple factors including, but not limited to site of administration, extent of disease, and method of administration. For example, an administration directly into the joint of a subject suffering from OA will typically contain a smaller number of cells than an administration of the cells into the bloodstream. The dose of cells disclosed herein can be repeated, depending on the patient's condition and reaction, at time intervals of days, weeks or months as determined necessary by a treating physician or other healthcare professional.

[0114] Compositions according to the present invention may be formulated for administration in fluid or liquid form for injection, or as part of a gel suitable for application to bone or other tissue surrounding the fracture. In certain embodiments where OCR stem cells are being used for treatment of bone fracture, administration is preferably in a therapeutically effective amount, this being sufficient to improve healing of the bone fracture compared to a corresponding untreated fracture or to a fracture treated with MSCs obtained from culture in control conditions. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the fracture. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and will typically take account of the nature of the fracture, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Single or multiple administrations of OCR stem cells doses may be administered in accordance with the guidance of the prescribing medical practitioner. Purely by way of example, OCR stem cells may be delivered in dosages of about 10-10,000,000 cells. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

[0115] OCR stem cells may be used to treat bone fracture alongside other treatments, such as administration of pain relieving or anti-inflammatory medicaments, immobilization and setting of the bone, e.g. immobilizing the injured limb in a plaster cast, surgical intervention, e.g. to re-set a bone or move a bone to correct displacement, angulation or dislocation. If surgery is required OCR stem cells may be administered directly to (e.g. applied to) the fracture during the surgical procedure.

[0116] Therapeutic compositions and medicaments of the invention may take the form of a biomaterial that is coated and/or impregnated with OCR stem cell. An implant may be formed from the biomaterial and be surgically implanted to assist in bone growth, regeneration, restructuring and/or re-modeling.

[0117] In other embodiments, OCR stem cells may be applied to implants to accelerate new bone formation at a desired location. The biomaterial may be coated or impregnated with OCR stem cells. Coating or impregnating may comprise contacting the OCR stem cells with the biomaterial such that they are allowed to be adsorbed and/or absorbed onto and/or into the

biomaterial. Coating may comprise adsorbing the OCR stem cells onto the surface of the biomaterial. Coating or impregnation of the biomaterial may involve seeding OCR stem cells onto or into the biomaterial. The biomaterial should allow the coated or impregnated OCR stem cells to be released from the biomaterial when administered to or implanted in the subject. Biomaterial release kinetics may be altered by altering the structure, e.g. porosity, of the biomaterial. Biomaterials coated or impregnated with ORC stem cells may improve the quality of life of a patient.

[0118] In other embodiments, the biomaterial provides a scaffold or matrix support. The biomaterial may be suitable for implantation in tissue, or may be suitable for administration (e.g. as microcapsules in solution). The implant should be biocompatible, e.g. non-toxic and of low immunogenicity (most preferably non-immunogenic). The biomaterial may be biodegradable such that the biomaterial degrades as wound healing occurs, ultimately leaving only the regenerated bone in situ in the subject. Alternatively a non-biodegradable biomaterial may be used, e.g. to guide bone regeneration over a large discontinuity and/or to act as a structural support during bone healing, with surgical removal of the biomaterial being an optional requirement after successful wound healing.

[0119] The matrix configuration can be dependent on the bone tissue that is to be produced. Preferably the matrix is a pliable, biocompatible, porous template that allows for target tissue growth. The matrix can be fabricated into structural supports, where the geometry of the structure is tailored to the application. The porosity of the matrix is a design parameter that influences cell introduction or cell infiltration. The matrix can be designed to incorporate extracellular matrix proteins that influence cell adhesion and migration in the matrix. Biomaterials may be soft and/or flexible, e.g. hydrogels, fibrin web or mesh, or collagen sponges. A "hydrogel" is a substance formed when an organic polymer, which can be natural or synthetic, is set or solidified to create a three-dimensional open-lattice structure that entraps molecules of water or other solutions to form a gel. Solidification can occur by aggregation, coagulation, hydrophobic interactions or cross-linking. Alternatively biomaterials may be relatively rigid structures, e.g. formed from solid materials such as plastics or biologically inert metals such as titanium. The biomaterial may have a porous matrix structure which may be

provided by a cross-linked polymer. The matrix is preferably permeable to nutrients and growth factors required for bone growth.

[0120] Matrix structures may be formed by crosslinking fibers, e.g. fibrin or collagen, or of liquid films of sodium alginate, chitosan, or other polysaccharides with suitable crosslinkers, e.g. calcium salts, polyacrylic acid, heparin. Alternatively scaffolds may be formed as a gel, fabricated by collagen or alginates, crosslinked using well established methods known to those skilled in the art.

[0121] Suitable polymer materials for matrix formation include, but are not limited by, biodegradable/bioresorbable polymers which may be chosen from the group of: agarose, collagen, fibrin, chitosan, polycaprolactone, poly(DL-lactide-co-caprolactone), poly(L-lactide-co-caprolactone-co-glycolide), polyglycolide, polylactide, polyhydroxyalcanoates, co-polymers thereof, or non-biodegradable polymers which may be chosen from the group of: cellulose acetate; cellulose butyrate, alginate, polysulfone, polyurethane, polyacrylonitrile, sulfonated polysulfone, polyamide, polyacrylonitrile, polymethylmethacrylate, co-polymers thereof.

[0122] A matrix with a high porosity and an adequate pore size can provide for increased cell introduction and diffusion throughout the whole structure of both cells and nutrients. Matrix biodegradability can provide for absorption of the matrix by the surrounding tissues (e.g., after differentiation and growth of bone tissues from progenitor cells) and can eliminate the necessity of a surgical removal. The rate at which degradation occurs should coincide as much as possible with the rate of tissue formation. Thus, while cells are fabricating their own natural structure around themselves, the matrix can provide structural integrity and eventually break down leaving the neotissue, newly formed tissue which can assume the mechanical load. Injectability is also preferred in some clinical applications. Suitable matrix materials are discussed in, for example, Ma and Elisseeff, ed. (2005) *Scaffolding in Tissue Engineering*, CRC, ISBN 1574445219; Saltzman (2004) *Tissue Engineering: Engineering Principles for the Design of Replacement Organs and Tissues*, Oxford ISBN 019514130X.

[0123] The biomaterial can be supplemented with additional cells. For example, one can "seed" the biomaterial with feeder cells, which may be useful for supporting growth and maintenance of the OCRs. The subject to be treated may be any animal or human. The subject is preferably

mammalian. In some embodiments the subject is a human. In other embodiments the subject is an animal, more preferably a non-human mammal. In certain embodiments, non-human mammals include rabbits, guinea pigs, rats, mice or other rodents (including any animal in the order Rodentia), cats, dogs, pigs, sheep, goats, cattle (including cows or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primates. The subject may be male or female. The subject may be a patient.

[0124] The present teachings include methods for optimizing the density of OCR stem cells (and their lineage derivatives) so as to maximize the regenerative outcome of a bone tissue. Cell densities in a matrix can be monitored over time and at end-points. Tissue properties can be determined, for example, using standard techniques known to skilled artisans, such as histology, structural analysis, immunohistochemistry, biochemical analysis, and mechanical properties. As will be recognized by one skilled in the art, the cell densities of progenitor cells can vary according to, for example, progenitor type, tissue or organ type, matrix material, matrix volume, infusion method, seeding pattern, culture medium, growth factors, incubation time, incubation conditions, and the like.

F. Kits

[0125] Also provided are kits. Such kits can include a therapeutic composition described herein and, in certain embodiments, instructions for administration. Instructions may be printed on paper or other substrate, or may be supplied as an electronic-readable medium, such as a floppy disc, mini-CD-ROM, CD-ROM, DVD-ROM, Zip disc, videotape, audio tape, and the like. Detailed instructions may not be physically associated with the kit; instead, a user may be directed to an Internet web site specified by the manufacturer or distributor of the kit. Such kits can facilitate performance of the methods described herein. When supplied as a kit, the different components of the composition can be packaged in separate containers and admixed immediately before use. Components include, but are not limited to OCR stem cells, culture media, and matrix or scaffold materials, as described herein. Such packaging of the components separately can, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the composition. The pack may, for example, comprise metal or plastic foil such as a blister pack. Such packaging of the components separately can also, in certain instances, permit long-term storage without losing activity of the components.

[0126] Kits may also include OCR stem cells in a container with or without other components such as water, media, growth factors etc. Containers may include test tubes, vials, flasks, bottles, syringes, bags or pouch, and the like. Containers may have a sterile access port, such as a bottle having a stopper that can be pierced by a hypodermic injection needle. Other containers may have two compartments that are separated by a readily removable membrane that upon removal permits the components to mix. Removable membranes may be glass, plastic, rubber, and the like.

5. Examples

[0127] The invention is illustrated herein by the experiments described by the following examples, which should not be construed as limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. Those skilled in the art will understand that this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will fully convey the invention to those skilled in the art. Many modifications and other embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Although specific terms are employed, they are used as in the art unless otherwise indicated.

Summary of Experimental Results

[0128] The following is a summary of results of experiments described in the Examples of this application:

- *Grem1* expression identifies a new bone, cartilage and stromal stem cell.
- *Grem1* cells are endogenous OCR stem cells.
- OCR stem cells are distinct from *Nes-GFP*⁺ MSCs.
- *Grem1* expression also defines intestinal connective tissue (reticular) stem cells (iRSCs).
- *Grem1*⁺ OCR stem cells contribute to fracture repair.

- A new model of skeletogenesis and intestinal mesenchymal homeostasis is established.

Example 1: Materials and Methods

Mice

[0129] The following lines were used: Nes-GFP (Mignone et al., 2004), Nes-CreERT² (Dranovsky et al., 2011), Grem1-LacZ (Khokha et al., 2003), Acta2-RFP (Magness et al., 2004), R26-LSL-ZsGreen (Madisen et al., 2010), R26-LSL- TdTomato (Madisen et al., 2010), R26-LSL-mT/mG (Muzumdar et al., 2007), 2.3ColGFP (Kalajzic et al., 2002), R26-LSL-Confetti (Snippert et al., 2010), and R26-LSL-DTA (Voehringer et al., 2008) (Table 1B). The R26-LSL- mT/mG was used in the intestine to better appreciate intestinal architecture, but for the bone marrow, either the R26-LSL-ZsGreen or the R26-LSL- TdTomato was used to enable the addition of a second reporter, such as Nes-GFP, 2.3colGFP or Acta2-RFP. We generated the Grem1-CreERT^T transgenic by BAC recombineering (clone RP24-317C19), as previously described (Sharan et al., 2009). The recombineering primers amplified the CreERT^T-pA-fNf cassette with 60 bp homology arms upstream and downstream of the Grem1 translational start site in exon 2 (Table 1). We generated three founder lines. Line 3 displayed the greatest recombination following adult tamoxifen induction, and it was backcrossed six generations to C57BL/6J. All experiments were performed according to the guidelines of the Institute of Comparative Medicine at Columbia University.

Marrow Stromal Cell Isolation

[0130] Long bones of the arms and legs were harvested and gently disrupted using a mortar and pestle, in PBS with 2% FBS and 1 mM EDTA. The bone and all of the liberated bone marrow were collected and digested in 0.25% collagenase type I (Worthington, Lakewood, NJ, USA, LS004196) in PBS with 20% FBS, for cell culture or flow cytometry.

Clonal In Vitro Bone Marrow MSC Culture, Clonogenicity Assays, and Differentiation

[0131] Marrow stromal cells were plated at clonal density and cultured for 14 days in aMEM + 10% defined MSC FBS + 1% penicillin/streptomycin. The total number of colonies, defined as R50 cells, was stained with Giemsa. The number of clones were reported as (CFU-Fs)/1,000

cells plated. For differentiation, single recombined clones were isolated using cloning cylinders and then expanded and split for differentiation using Invitrogen StemPro differentiation products into adipocytes, chondrocytes, and osteoblasts. All in vitro differentiation reported in this study is clonal.

Fracture Studies

[0132] Adult *Grem1-creERT^T;R26-LSL-TdTomato;2.3ColGFP* mice were induced with tamoxifen with a 1 week washout period before fracture. Unilateral femoral osteotomy was internally fixed by an angiocatheter. Femurs were harvested at 7 days. For the fracture transplantation, a single *Grem1*-derived clone (after adult in vivo induction) was expanded in vitro and then 500×10^6 cells were mixed with a HyStem-C(TM) Hydrogel Kit (Glycosan) and injected around the fracture sites of the recipient wild-type mice. The fractured bones were harvested at 7 days. Some of the fracture callus was recultured to recover the donor *Grem1*⁺ cells. The fractures were imaged by X-ray and a Kodak In Vivo Multispectral Imaging System FX (carestream Health) specific for TdTomato fluorescence.

Tissue-Engineered Small Intestines

[0133] Organoid units were harvested from 3-week-old, P1 tamoxifen-induced *Grem1-creERT^T;R26-LSL-TdTomato* donor mice and transplanted into 8 wild-type adult C57BL/6 mice. The procedure was otherwise performed as previously described (Levin et al., 2013) with the TESIs harvested at 4 weeks post-implantation for analysis.

Tamoxifen induction of mice

[0134] Tamoxifen for adult induction of creERT lines was administered at 6 to 8 weeks of age. Induction schedule for intestine was one 6mg dose of tamoxifen dissolved in 300μL of corn oil administered by gastric gavage. For the bone marrow 4 x 6mg doses of tamoxifen were administered alternate days by gastric gavage. For perinatal induction, the pups were injected subcutaneously with 2mg of tamoxifen dissolved in corn oil. The embryonic induction was 2mg of tamoxifen administered by oral gavage to pregnant dams at E13.5.

Flow cytometry

[0135] For all flow cytometry and FACS the cells were blocked and then incubated with primary antibodies from BioLegend and BD Biosciences following standard procedures (Table 2). Flow cytometry and FACS was performed on a BD FACS Aria cell sorter.

In Situ Hybridization

[0136] Single whole-mount in situ hybridization was performed as previously described (Brent et al., 2003). Briefly, embryos were fixed in 4% PFA, dehydrated into Methanol and bleached. DIG-labeled antisense probes (~300- 600 ng/ml) were hybridized at 70°C, detected with α -DIG-AP (1:2000, Roche), and developed using BM Purple (Roche). Grem1 probe was a generous gift from Richard Harland.

Microarray

[0137] n=3 adult (6-8 weeks) Grem1-creERT;R26-LSL-TdTomato mice were induced and bone marrow sorted by FACS with the non-recombined CD45/CD31/Ter 119 triple negative population compared to the Grem1+ cells. The extracted RNA was amplified using the Nugen single direct kit. Data from the hybridized chips were scanned and analyzed using Bioconductor and R software (Gentleman et al., 2004; Ihaka and Gentleman, 1996). All chips passed recommended QC tests (Bolstad et al., 2005), Normalization was performed using GCRMA (Wu and Irizarry, 2005; Wu et al., 2004) and statistical analysis was performed using Limma (Smyth, 2004). A cutoff of a Benjamini-Hochberg False Discovery Rate (Benjamini and Hochberg, 1995), $fdr < 0.05$, was used found. The array data was deposited in the Gene Expression Omnibus (GSE57729)(Barrett et al., 2005). Pathway Analysis was performed with PathwayGuide (Tarca et al., 2009)

Analysis of clonal Confetti labeling in the bone

[0138] We estimated the probability that patches of adjacent identically-colored cells (“clones”) where not in fact clonally-derived, but instead due to the merging of multiple independently initiated clones that by chance bore the same confetti color. To do this, we constructed a Monte Carlo simulation of clone labeling in the growing bone. We assumed that the analyzed area of the bone growth plate at P1 could be reasonably described by a square-lattice, consisting of the

approximately 400 cells (which subsequently grow to form the approximately 1000 cells that constitute the analyzed area of the growth plate at 6 weeks of age). The transformation efficacy at P1 was measured to be 94/439 cells (approx. 21%), but we note that only 8 clones were observed at 6 weeks implying the fraction of labeled clones that survive to 6 weeks is 8/94 (approx. 9%). To initiate each Monte Carlo simulation we randomly labeled 2% of cells (e.g. the transformation efficiency x survival fraction) as red, blue or yellow with equal probability according to the observed proportions of each confetti-color (green clones were never observed in practice). Clones were observed to grow to an average of 8 cells by 6 weeks (std dev: 6.2 cells), thus we assumed that two clones within a distance of 3 cells (approximately twice the radius of the average clone size) would collide (merge) in the growing bone. For each labeled cell, we then computed any collisions, and recorded the proportion of the resulting "clones" that were composed of two or more independently labeled cells of the same color. All simulations were performed in the R statistical computing environment. The simulations suggest that 9% of patches of adjacent uniformly colored cells actually had a polyclonal origin, thus >90% were monoclonal.

MicroCT analyses

[0139] MicroCT was performed on a Quantum FX Micro-CT (Perkin-Elmer). The 3D microCT images were imported to image analysis software (ImageJ, National Institutes of Health, Bethesda, MD). A heuristic algorithm was used to eliminate non-bone voxels. Bone volume was estimated by multiplying the total bone voxel counts in the region of interest (e.g. total field or left femur) after segmentation by the volume per voxel.

Histology

[0140] Femoral sections were processed in usual fashion and sectioned using either a Tungsten blade and the CryoJane tape transfer system or following decalcification before conventional sectioning. For the *Grem1* in situ hybridization on adult small intestine we used an ACD RNAscope® FFPE reagent kit specific for Grem1, using manufacturer's instructions.

Transmission Electron Microscopy

[0141] To define the anatomical relationship between the Grem1+ iRSC-derived periepithelial sheath and the epithelium of the small intestine, fragments from the jejunum of a 6-week-old Grem1-creERT;R26-LSL-TdTomato mouse induced at P1 were fixed by 4% PFA and 0.1% glutaraldehyde. Specimens were embedded in 20% gelatin and 70 μ m sections were cut by vibratome. The sections were incubated for 30 minutes at room temperature in the blocking solution (10% normal goat serum in phosphate buffered saline (PBS)) and then with polyclonal rabbit antibodies against dsRed diluted (Table 2). Primary antibodies were located with biotinylated secondary antibodies and avidin coupled to horseradish peroxidase (HRP; Vector ABC elite kit) and visualized with 3-3'-diaminobenzine and glucose/glucose oxidase to generate the peroxide substrate. The sections were then dehydrated through a graded ethanol and examined with a JEOL 1200EX electron microscope.

Small intestinal lineage tracing

[0142] 6mg of tamoxifen was administered by oral gavage once to 6-8 week old mice, which were then sacrificed at increasing time points post tamoxifen including 24 hours, 1 month, 3 months, 6 months, 9 months, 12 months and 24 months. Three to 5 mice were sacrificed at each time point.

Isolation of Small intestinal CFU-Fs

[0143] Intestines from Grem1-creERT; R26-LSL-TdTomato mice were induced at P1, digested with collagenase VIII and dispase adapted from previous protocols (Manieri et al., 2012; Newberry et al., 1999). The cells were cultured in a 10cm dish containing 10mLs of DMEM with 10% FBS, 1% antibiotics with 100 μ L of 20mg/mL DNase I.

Statistics

[0144] All analyses were performed using Stata version 12 (StataCorp, College Station, Texas, USA) or Prism 6 (GraphPad software Inc.).

Example 2. Generating a Specific Marker of Skeletal Stem Cells

[0145] To select a specific MSC marker in the bone and intestine, we considered human gene-expression arrays from bone marrow, intestine, and peritumoral mesenchyme (Delorme et al.,

2009; Kosinski et al., 2007; Sneddon et al., 2006). Gremlin 1 (*Grem1*), identified from these studies, is a secreted antagonist of bone morphogenetic protein (Bmp) -2, -4, and -7 and a VEGFR2 agonist (Hsu et al., 1998; Mitola et al., 2010). *Grem1* is important in normal skeletal and renal development and homeostasis (Canalis et al., 2012; Khokha et al., 2003; Michos et al., 2004). Furthermore, overexpression of *Grem1* interrupts normal intestinal function and has been linked to intestinal cancer (Jaeger et al., 2012). *Grem1* expression identified the most clonogenic fraction of marrow stromal cultures (Quante et al., 2011). In the present study, it was confirmed that expression of *Grem1* was increased in undifferentiated mesenchymal cultures compared to endogenous bone marrow mesenchyme (FIG. 1A–FIG. 1C). To extend these findings in vivo, a tamoxifen-inducible BAC transgenic *creER^T* line specific for *Grem1* expression (*Grem1-creER^T*, was generated. (FIG. 1D– FIG. 1F, Table 1). The *Grem1-creER^T* BAC transgenic line was crossed to different reporters (such as *R26-LSL-TdTomato* and *R26-LSL-ZsGreen*) and the *R26-LSL-diphtheria toxin subunit A (DTA)* line to allow lineage tracing and functional ablation of specific mesenchymal cells, respectively (See Tables 1B and 1C for summary of transgenic lines).

Example 3. Grem1⁺ Cells Are Distinct from, and More Clonogenic than, Nes-GFP⁺
[0146] MSCs Tamoxifen induction of adult *Grem1-creER^T;R26-LSL-TdTomato* mice (Figure 1A) resulted in recombination in and expression of the TdTomato reporter (red fluorescent protein) in a rare and exclusively mesenchymal population of bone marrow cells (0.0025% of all single, live, nucleated cells after collagenase digestion [95% confidence interval (CI) 0.0022–0.0028]). In this experiment and elsewhere in the paper, we defined skeletal mesenchyme as triple negative for CD45[−]Ter-119[−]CD31[−] in enzymatically digested bone and bone marrow cells. CD45 characterizes most hematopoietic cells with the exception of maturing erythroid cells, which are marked by Ter-119. CD31 was used to exclude endothelial cells (Park et al., 2012) (Table 1D). The CD45-negative, Ter-119-negative, and CD31-negative fraction of bone marrow defines the nonendothelial, nonhematopoietic compartment that contains putative skeletal stem cells. Many *Grem1-creER^T*⁺ cells, identified by a recombined fluorescent reporter gene shortly after tamoxifen administration (hereafter referred to as *Grem1⁺* cells), were immediately adjacent to the growth plate and trabecular bone (FIG. 1B,

FIG. 1C, and FIG. 5B).

[0147] To determine the overlap between *Grem1*⁺ and other reported CFU-F populations, *Grem1-creERT*^T were crossed to *Nes-GFP*; *R26-LSL-TdTomato* (*Grem1*⁺ cells and their progeny were red, and *Nes-GFP*-expressing cells were green) and to *Acta2-RFP*; *R26-LSL-ZsGreen* (*Grem1*⁺ cells and their progeny were green, and *Acta2-RFP*-expressing cells were red) (Grcevic et al., 2012; Méndez-Ferrer et al., 2010). Adult *Grem1*⁺ cells did not express *Nes-GFP* (FIG. 1B and FIG. 1D), and only a minority of *Grem1*⁺ cells expressed *Acta2-RFP* (mean 5.9% of *Grem1*⁺ were *Acta2*⁺; FIG. 9A). The *Grem1*⁺ population was more clonogenic than *Nes-GFP* or *Acta2-RFP* cells, even after depletion of contaminating nonmesenchymal lineages (FIG. 1D– FIG. 1G and FIG. 9B). *Grem1*⁺ CFU-Fs did not initially express *Acta2-RFP*, but during culture, the *Grem1*⁺ progeny differentiated into *Acta2-RFP*-expressing myofibroblasts (these cells were yellow, expressing both *ZsGreen* and *Acta2-RFP*; FIG. 9C). Importantly, the proportion of CFU-Fs was diminished within the *Grem1*⁺ descendants expressing *Acta2-RFP*, suggesting that *Acta2* expression marks a more differentiated cell less capable of clonogenic growth (FIG. 9D). *Grem1*⁺ cells, however, never gave rise to *Nes-GFP*-expressing cells, either in adherent culture or in vivo (10 months tracing after adult tamoxifen induction of *Grem1-creERT*^T; *R26-LSL-TdTomato*; *Nes-GFP* mice; FIG. 9E).

[0148] For the clonal differentiation experiments (FIG. 1), adult *Grem1-creERT*^T; *R26-LSL-ZsGreen*; *Acta2-RFP* and *Grem1-creERT*^T; *R26-LSL-TdTomato* mice were administered tamoxifen by oral gavage at 6–8 weeks of age. Bones were processed as described (see Experimental Procedures), and the fluorescent *Grem1*⁺ cells were sorted by fluorescence-activated cell sorting (FACS) and plated at clonal density < 2,500 cells/10 cm dish. Clones (i.e., isolated clusters of > 50 cells at 10–14 days) were harvested using a cloning cylinder and expanded into individual wells for differentiation. 19 clones were evaluated after in vitro differentiation. Single clones gave rise to osteoblasts (defined by alizarin red [FIG. 1H] or alkaline phosphatase staining in 84% of clones, [FIG. 9F and FIG. 9G]), chondrocytes (100% of clones confirmed by toluidine blue staining), and myofibroblasts on the basis of coexpression

of the *Acta2-RFP* transgene (100%, FIG. 1H– FIG. 1J). In contrast, the *Grem1*⁺ cell-derived clones showed little capacity for adipocytic differentiation (defined by oil red staining, Figure 1K, 0 of 19 single clones). Polyclonal cultures from sorted *Grem1*⁺ cells also failed to differentiate into adipocytes.

[0149] A substantial proportion (40%) of *Grem1*⁺ cells, in addition to being triple negative for CD45[−]Ter-119[−]CD31[−], were also positive for CD105, a well-established marker of bone marrow CFU-Fs (Park et al., 2012). In contrast, less than 2% of the *Grem1*-negative cells were CD45[−]CD31[−]Ter-119[−]CD105⁺. *Grem1*⁺ cells, however, expressed lower levels of CD140a and Sca-1 (FIG. 2A– FIG. 2F). The *Grem1*⁺ population was enriched for CD45[−]CD31[−]Ter-119[−]CD105⁺ cells, a subpopulation previously reported to contain all mouse bone marrow CFU-Fs (Park et al., 2012). It followed that *Grem1*⁺ cells were also enriched for CFU-Fs compared to *Grem1*-negative fractions (FIG. 10A and FIG. 10B). It is worth noting that we used standard adherent cell culture conditions (aMEM with 10% FBS), whereas other studies have used 20% FBS, hypoxic conditions, and a ROCK inhibitor, which enhance the recovery of CFU-Fs (Zhou et al., 2014). Thus, the exact CFU-F efficiencies reported in our study may not be directly comparable to other reports.

[0150] Gene-expression microarray of *Grem1*⁺ versus *Grem1*-negative mesenchymal (CD45[−]CD31[−]Ter-119[−]) cells revealed 1,426 differentially expressed genes (false discovery rate [FDR] < 0.05). The *Grem1*⁺ population had significantly higher expression of many osteoblast (*Sp7*), chondrocyte (*Acan*), pericytic (*Cpsg4*, *Fap*), and putative stem cell genes (*Klf4*), all of which were confirmed by qPCR (FIG. 2G– FIG. 2M, , and FIG. 10C and FIG. 10E). *Grem1*⁺ cells did not differentially express *Nes* (FIG. 2H) or other genes typical of the perisinusoidal mesenchymal niche.

[0151] To determine the signaling pathways that were activated in the *Grem1*⁺ cells, we investigated candidate pathways previously reported to be relevant in MSC differentiation, such as the BMP, TGF- β , FGF/PDGF, and VEGF pathways (Gerber et al., 1999; Ng et al., 2008). Although all of these pathways were statistically significant (<2.2 $\times 10^{-16}$ by the c2 test),

only the genes in the Bmp-activating pathway were consistently increased. *Bmp2*, *Bmp5*, *Bmp6*, the Bmp receptor *Acvr1*, and the BMP signaling target gene *Id2* were all upregulated in *Grem1*⁺ versus *Grem1*⁻ negative mesenchymal cells (FIG. 10F and Table 1E). To support the role of BMP signaling in the *Grem1*⁺ population, we performed qPCR on 23 clones derived from *Grem1*⁺ bone marrow cells. *Id2* was expressed in 100% of clones, and *Bmp2* was expressed in 91% of clones. Furthermore, using flow cytometry, pSmad1,5, a marker of BMP signaling, was detected in 37.5% of *Grem1*⁺ versus 5.8% of *Grem1*-negative freshly sorted cells (Data S1G, p = 0.004). In contrast, genes identified in the TGF- β , FGF/PDGF, and VEGF pathways were found to be both activators and repressors and so did not generate a coherent signal constituting pathway activation (FIG. 10F, Data 1H, and FIG. 10I). We also used an unbiased analysis in which all differentially expressed genes in *Grem1*⁺ versus *Grem1*⁻ negative cells (fdr < 0.05) were evaluated against the KEGG and Reactome databases. Pathways with a gamma fdr < 0.05 are included in Table 1F (KEGG) and Table 1G (Reactome). The top 3 significant KEGG pathways, the ECM-receptor interaction, PI3K-Akt signaling, and focal adhesion pathways, were all activated according to PathwayGuide's statistical criterion. Differentially expressed genes from these pathways are given in Table 1H–Table 1I. Many of the genes upregulated in these pathways are involved in differentiation into bone and cartilage and include the following: chondroadherin, cartilage oligomeric matrix protein, fibroblast growth factors, collagens, integrins, and cyclins. Taken together, BMP signaling along with ECM-receptor interaction, PI3K-Akt signaling, and focal adhesion pathways were all significantly activated in the *Grem1*⁺ cells and are likely to be important either in determining or as a consequence of the *Grem1*⁺ cells' osteochondral lineage potential.

[0152] The expression profile of *Grem1*⁺ cells was enriched for genes implicated in bone and cartilage, rather than adipocytic, differentiation (FIG. 10J; FIG. 10L– FIG. 10N). Furthermore, *Grem1*⁺ cells, and their derivative clones, expressed active inhibitors of adipogenesis (e.g., *Nr2f2*; FIG. 10K; Xu et al., 2008). The exact molecular explanation for the more restricted mesenchymal repertoire of *Grem1*⁺ cells, however, remains to be confirmed.

[0153] Further qPCR analysis of 23 *Grem1*⁺ cell-derived clones confirmed the mesenchymal homogeneity of *Grem1* cells and their derivative clones (FIG. 10L). Interestingly, 100% of

the *Grem1*⁺ clones expressed both *Nes* and *Grem1*, and *Grem1* transcripts were also detectable within polyclonal *Nes-GFP* cultures. Thus, endogenous *Grem1* and *Nes* expression are not as mutually exclusive as their respective transgenes in vivo. Finally, *GREM1* is also expressed in human MSCs. We examined three distinct human MSC lines derived from normal bone marrow, normal synovium, or inflamed synovium. All lines expressed *GREM1* (FIG. 10M).

Grem1 Cells Are Endogenous OCR Stem Cells

[0154] *Grem1-creER^T;R26-LSL-ZsGreen;Acta2-RFP* mice were induced with perinatal tamoxifen (postnatal day [P] 1, FIG. 3A). In these mice, all *Grem1*⁺ cells and their subsequent progeny were labeled by green fluorescence, and any cells expressing *Acta2* were marked by red fluorescence. Twenty-four hours after P1 induction, *Grem1*⁺ (green) cells were present within the primitive mesenchyme and the primary spongiosa of the femur. In contrast, the *Acta2*-expressing (red) stromal cells were localized within the bone marrow (FIG. 3B). By P5, *Grem1*⁺ cells had differentiated into columns of chondrocytes (FIG. 3C and FIG. 3D, green) as well as spindle-shaped stromal cells immediately inferior to the developing growth plate (FIG. 3C and FIG. 3E).

[0155] By using *Grem1-creER^T;R26-LSL-TdTomato;2.3colGFP* mice, one can track *Grem1*⁺ cells and their progeny by red fluorescence, and osteoblasts are marked by green fluorescence. The *2.3colGFP* mouse is a transgenic line in which GFP expression, driven by a short 2.3 kb promoter element from the rat collagen 1a1 gene, has been used to identify committed osteoblasts (Kalajzic et al., 2002). After 6 weeks, the P1-labeled *Grem1*⁺ cells had differentiated into reticular marrow stromal cells (red), chondrocytes (red), and osteoblasts (yellow), all concentrated within the peritrabecular bone area (FIG. 3F–FIG. 3I). Many of the reticular marrow stromal cells anatomically spanned perivascular and endosteal regions and were CD105⁺ by immunostaining (FIG. 10A). As early as 4 weeks (P28) following P1 induction,

[0156] *Grem1*⁺ cells give rise to approximately 64% of the bone and 50% of the chondrocytes within the metaphysis and epiphysis, albeit with little contribution to diaphyseal bone (FIG. 10B). To prove that the fluorescent cells represented clonal populations, we generated *Grem1-creERT^T;R26-Confetti* mice and again induced at P1. Single-color clones of chondrocytes and marrow stromal cells were present by 6 weeks, confirming single-cell multipotentiality (FIG. 3J and FIG. 3K). Our Monte Carlo simulation confirmed that the majority (>90%) of patches of

adjacent identically colored cells (“clones”) were likely to be monoclonal in origin (see Experimental Procedures).

[0157] Approximately 12 months after adult (6–8 weeks, FIG. 3L) tamoxifen induction of *Grem1-creERT;R26-LSL-TdTomato* mice (and *Grem1-creERT;R26-LSL-ZsGreen*, mice), we found that *Grem1*⁺ cells had differentiated into columns of chondrocytes (FIG. 3M and FIG. 3N), articular cartilage (FIG. 10C), reticular marrow stromal cells (Figure 3O), periosteal cells (Figure 3P), diaphyseal osteoblasts (FIG. 10D), and osteocytes (Figure 3P). No adipocytes, either in the femurs (FIG. 10E) or in the vertebral bodies (FIG. 10F), were derived from adult *Grem1*⁺ cells, even at 1 year after tamoxifen induction. Fluorescently labeled *Grem1*⁺ cells harvested from the bone marrow after 1 year could still self-renew in vitro. The harvested *Grem1*⁺ cells in culture formed large colonies, which were clonally expanded (using cloning cylinders) to prove that they retained clonal in vitro multi-potentiality, even 12 months after adult tamoxifen induction (FIG. 10G). Again, in vitro differentiation was skewed to osteoblast and chondrocyte (FIG. 10G–FIG. 10J), with little in vitro adipogenesis (FIG. 10H). These results indicate that expression of *Grem1* also identified adult multipotent stem cells.

[0158] To confirm that *Grem1*⁺ cells were functional, postnatal skeletal stem cells, *Grem1-creERT;R26-LSL-ZsGreen;R26-LSL-DTA* mice and *Grem1-creERT;R26-LSL-ZsGreen* littermate and related controls were generated. In these mice, *Cre*-mediated excision of a STOP signal leads to the expression of the Diphtheria toxin (DTA) and thus ablation of *Grem1*-expressing cells. We administered four daily doses of 2 mg subcutaneous tamoxifen starting at P9 and measured total body and left femoral bone volume by microcomputed tomography (CT) at P23 (Quantum FX MicroCT, Perkin-Elmer; FIG. 11A–FIG. 11C). *Grem1* cells were incompletely ablated, although reduced, as assessed by the proportion of *ZsGreen*-expressing cells in the bone marrow (FIG. 11A). The DTA⁺ mice were significantly smaller than the controls (FIG. 11B). There was a significant difference in femoral volume between the groups and a trend toward reduced total bone volume in the DTA versus the control mice (FIG. 11C).

[0159] It was noted that the major site of *Grem1*-driven recombination at P23, after P9 induction, was within the femoral epiphysis (nearly 60% of the epiphyseal bone was labeled). Therefore, we examined anatomically comparable sections in *Grem1* DTA versus control mice and measured the fraction of mineralized bone in the femoral epiphysis. Here too, the trabecular bone

fraction was significantly reduced in DTA mice versus control mice (FIG. 11D). This suggested a functional role of *Grem1*⁺ cells in postnatal skeletogenesis. We acknowledge, however, that ablation of extra-skeletal *Grem1*⁺ cells may have indirectly contributed, at least in part, to the impaired skeletogenesis.

[0160] The expression of *Grem1*, *Nes*, *Runx2*, and *Sox9* was measured by whole-mount in situ hybridization during the earliest stages of hind limb bud development, i.e., embryonic day (E) 9.5, E10.5, E11.5, and E12.5 (FIG. 4A). *Grem1* was expressed at the onset of hind limb development, E9.5. *Nes* was not expressed within the hind limb at these stages. To confirm that *Grem1* marked a multipotent mesenchymal stem/progenitor cell during development, tamoxifen was administered to pregnant *Grem1-creERT*;*R26-LSL-TdTomato* dams at E13.5. Consistent with the in situ hybridization findings, *Grem1* was expressed within much of the primitive hind limb mesenchyme within the embryos and gave rise to almost all of the cells within the primitive mesenchyme and the primary spongiosa by E21 (FIG. 11E and FIG. 11F). Taken together, these results confirm that *Grem1* expression marked a new, endogenous skeletal stem cell, in development and adulthood, which lacked any significant capacity for adipogenesis. As a result, it does not meet the minimal criteria for MSCs (Dominici et al., 2006). Thus, we named these *Grem1*⁺ stem cells as osteochondroreticular “OCR stem cells” in reference to the earlier concept of the osteochondroprogenitor (Ducy et al., 1997).

Example 4. Perisinusoidal Nes-GFP⁺ Cells May Not Be Skeletal Stem Cells during Early Life

[0161] The previously published *Nes-cre* and *Nes-creERT* lines may not reliably identify the perisinusoidal *Nes-GFP* cells that are purported to be endogenous MSCs (Ding et al., 2012; Méndez-Ferrer et al., 2010). Thus, we used a different transgenic *Nes-creERT* line in an attempt to better understand the lineage potential of *Nes-GFP*⁺ perisinusoidal MSCs (Dranovsky et al., 2011). Compared to previously reported *Nes* reporter mouse lines, the transgenic *Nes-creERT* line used here had a different *Nes* regulatory sequence directing the expression of *creERT* (Dranovsky et al., 2011). Following P1 tamoxifen induction of *Nes-creERT*;*R26-LSL-TdTomato*;*Nes-GFP* mice (FIG. 4B), *Nes-creERT* recombined *R26-LSL-TdTomato* in approximately 4% of all bone marrow *Nes-GFP* cells by 6–8 weeks (FIG. 4C). This included metaphyseal (FIG. 4D), periarteriolar (FIG. 4E), and perisinusoidal *Nes-GFP* cells (FIG. 4F–FIG. 4H). In keeping with previous perisinusoidal lineage-tracing studies, this *Nes-creERT*

line did not generate cartilage or trabecular bone by 6–8 weeks (Ding et al., 2012; Mizoguchi et al., 2014). The only osteochondral cells that were traced consisted of rare, isolated osteocytes embedded within the diaphyseal cortical bone (FIG. 4H). Our findings suggest that perisinusoidal cells, labeled by *Nes-GFP*, may not be the principal skeletal stem cells during development or early postnatal life (Ding et al., 2012; Mizoguchi et al., 2014; Zhou et al., 2014). It is quite possible, however, that the endogenous *Nes* gene or other *Nes*-transgenic lines could be expressed in postnatal skeletal stem/progenitor cells, but that these cells were not captured by our lineage-tracing strategy in young (<8-week-old) mice.

Example 5: Grem1⁺ OCR Stem Cells Contribute to Fracture Repair

[0162] Adult *Grem1⁺* cells do not overlap with *2.3colGFP⁺* osteoblasts. *Grem1⁺* cells are, however, adjacent to osteoblasts in vivo and during early adherent bone marrow stromal culture (FIG. 5B and FIG. 5C). Surgical fracture with internal fixation of the femur in *Grem1-creER^t;R26-LSL-TdTomato;2.3colGFP* mice (performed 1 week after adult induction with tamoxifen) (FIG. 5A and FIG. 5D) resulted in *Grem1⁺* OCR stem cell expansion and differentiation into *2.3colGFP*-positive (and osteocalcin-positive, FIG. 12D) osteoblasts and *2.3colGFP*-negative, *Sox9⁺* chondrocytes within the fracture callus (FIG. 5E–5H and FIG. 12A–FIG. 12C). The *Grem1⁺* OCR stem cell lineage, as defined by red fluorescence, contributed approximately 28% of osteoblasts (red and *2.3colGFP* green) and 14% of chondrocytes (defined by *Sox9* immunostaining and/or by hematoxylin and eosin staining [H&E] on serial sections) within the fracture callus. Next, we tested whether *Grem1⁺* cells could be transplanted into the fracture site.

[0163] A clonal population of *Grem1⁺* OCR stem cells was expanded and harvested. This clone, mixed with hydrogel, was applied to the fracture site at the time of injury and engrafted into the callus of the recipient wild-type mice (FIG. 5I and FIG. 5J). The transplanted cells differentiated into osteoblasts (alkaline phosphatase-expressing) within the fracture callus (FIG. 5K). OCR stem cells self-renewed within the callus and were recovered from the recipient animals and rapidly expanded again in fracture callus cultures (Figure 5L). The *Grem1⁺* OCR stem cells cultured from the fracture callus and expanded in vitro could be serially transplanted into a second fracture (FIG. 12E). Thus, *Grem1* expression identifies developmental

and adult, both physiological and reactive, endogenous OCR stem cells amenable to serial transplantation.

Example 6: Grem1 Expression also Defines a New iRSC

[0164] It was investigated whether *Grem1* could also mark extramedullary connective tissue stem cells. The small intestine was selected as our extramedullary organ of interest because the gut is known to contain multipotent mesenchymal stromal cells (Powell et al., 2011). It is worth emphasizing that we searched for a connective tissue stem cell within the lamina propria and not for an epithelial stem cell, such as that previously identified by *Lgr5* expression (Barker et al., 2007). Furthermore, the small intestine does not contain bone and cartilage, and thus it was not expected to find a bona fide OCR stem cell. Rather, testing for an organ-relevant connective tissue stem cell, sharing *Grem1* expression and the defining stem cell characteristics of self-renewal and multipotentiality was of interest. The connective tissue immediately beneath the intestinal epithelium is a mesenchymal sheath that invests the entire intestinal gland (Powell et al., 2011). Adult *Grem1* recombination (24 hr after 6 mg of tamoxifen by oral gavage, *Grem1-creER^T;R26-mT/mG*) identified single cells (*Grem1*⁺ = green) immediately beneath the epithelium at the junction between the small-intestinal crypt and villus, a region known as the intestinal isthmus (FIG. 6A and FIG. 6B). In situ hybridization confirmed *Grem1* expression within periepithelial isthmus cells, and these cells were also positive in the *Grem1-LacZ* knockin line (. FIG. 14A–FIG. 14F) (Khokha et al., 2003).

[0165] Single periepithelial *Grem1* cells divided slowly (BrdU incorporation over 1 month of continuous dosing; FIG. 14G). Over time, the fluorescently tagged *Grem1*⁺ cells gave rise to reticular, periepithelial mesenchymal lineages throughout both the crypt and villus sheaths (FIG. 6B–FIG. 6E). The *Grem1*⁺ cells differentiated into both *Acta2*-positive myofibroblasts (FIG. 6F) and *Acta2*- negative, but *Ng2*-positive, stromal cells (.FIG. 14H). *Grem1* cells gave rise to an intricate reticular network of cells that invested the entire gland (FIG. 6B– FIG. 6E,). The *Grem1* lineage was distinct from the closely associated *s100b* (FIG. 14I) and *Nes-GFP* positive periepithelial glial sheath (FIG. 14J) (Belkind-Gerson et al., 2013). The *Grem1*⁺ cell lineage was related to the overlying intestinal epithelium (FIG. 6G and FIG. 14K). After 6 to 9 months, intestinal *Grem1* cells had expanded to give rise to the mesenchymal sheath subjacent to the *Lgr5*⁺ crypt base columnar stem cell zone (FIG. 6B, FIG. 6C, and FIG. 6E) (Barker et

al., 2007). After 12 months, the cells had also renewed the periepithelial mesenchymal sheath to the tip of the villi (FIG. 6B). The *Grem1*⁺ cells at the isthmus self-renewed so that their fluorescently tagged lineage persisted across the entire crypt-villus axis for at least 2 years after adult tamoxifen induction (FIG. 14L). Perinatal induction at P1 led to accelerated intestinal mesenchymal tracing (at 6 weeks), providing further proof of the clonality of the *Grem1* lineage within the mesenchymal sheath (*Grem1-creER^T;R26-Confetti* mice; FIG. 14-M). The *Grem1* lineage also identified small-intestinal CFU-Fs (FIG. 14N). Similar patterns of lineage tracing were evident throughout the entire gastrointestinal tract, including the stomach (FIG. 14O). *Grem1* also identified a progenitor population within the skin (FIG. 14O). The exact nature of these cells, however, requires further study.

[0166] Extending the concept of connective tissue stem cells to extramedullary mesenchyme, however, requires that the model be rigorously validated in vivo. A protocol was adopted for generating tissue-engineered small intestines (TESIs) to test whether *Grem1*⁺ intestinal mesenchymal cells could be transplanted to generate the small-intestinal, periepithelial mesenchymal sheath within the recipient's TESI graft (Levin et al., 2013). *Grem1-creER^T;R26-LSL-TdTomato* donor small-intestinal organoid units were transplanted into the omentum of recipient wild-type mice. Only one or two *Grem1*⁺ cells were present within the harvested donor units (FIGs. 6H), but they were able to expand and restore the intestinal mesenchymal sheath in the TESI (FIGs. 6I and FIG. 14P). Thus, small-intestinal *Grem1*⁺ cells self-renew and clonally generate multiple distinct, compartment-relevant mesenchymal lineages and can be transplanted to recapitulate the periepithelial mesenchymal sheath, confirming that they are connective tissue stem cells. In this context, they are referred to as iRSCs. The term, iRSCs, reflects their morphology and the reticular network formed by these cells and in reference to their *Grem1*-expressing reticular counterparts in the bone marrow.

[0167] Although the present invention has been described in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred embodiments contained herein.

Table 1A

Gene	Purpose	Sequence (5'-3')/Sequence name (IDT descriptor)	
F-Grem1 recombineering primer	Recombineering	GATTTTTACTAAATAACTTTCTTATTGTCTGTG TCCCCCTCTCTTTGTCCTTTGTCTAGAATGTC CAATTTACTGACCGTACA	SEQ ID NO.: 1
R-Grem1 recombineering primer	Recombineering	GTTGGCAGTAGGGTCCCCAGGAGGAGAAGC AACGCTCCCACAGTGTATGCGGTGCGATTAT TATGTACCTGACTGATGAAGTT	SEQ ID NO.: 2
F- Grem1-creERT	Genotyping	CTG TGT CGA ATT ACT CAG TTT GAT G	SEQ ID NO.: 3
R- Grem1-creERT	Genotyping	AAT GTT GCT GGA TAG TTT TTA CTG C	SEQ ID NO.: 4
F- Grem1-LacZ	Genotyping	ATCCTCTGCATGGTCAGGTC	SEQ ID NO.: 5
R- Grem1-LacZ	Genotyping	CGTGGCCTGATTCATTCC	SEQ ID NO.: 6
F- R26-TdTomato or ZsGreen (the WPRE)	Genotyping	ACT GTG TTT GCT GAC GCA AC	SEQ ID NO.: 7
R- R26-TdTomato or ZsGreen (the WPRE)	Genotyping	CAA CAC CAC GGA ATT GTC AG	SEQ ID NO.: 8
F- Nes-GFP or 2.3ColGFP or R26-Confetti (the GFP)	Genotyping	GAG CTG AAG GGC ATC GAC TTC AAG	SEQ ID NO.: 9
R- Nes-GFP or 2.3ColGFP or R26-Confetti (the GFP)	Genotyping	GGA CTG GGT GCT CAG GTA GTG G	SEQ ID NO.: 10
F- Nes-CreERT	Genotyping	GCG GCA TGG TGC AAG TTG AAT	SEQ ID NO.: 11
R- Nes-CreERT	Genotyping	CGT TCA CCG GCA TCA ACG TTT	SEQ ID NO.: 12
F- R26-iDTA	Genotyping	ACC TGG TTA TGT AGA TTC CAT TCA A	SEQ ID NO.: 13
R- R26-iDTA	Genotyping	CAG AAG TAA GGT TCC TTC ACA AAG A	SEQ ID NO.: 14
<i>Acan</i>	qPCR	Mm.PT.56a.10174685	
<i>Angpt2</i>	qPCR	Mm.PT.56a.29139310	
<i>Bmp2</i>	qPCR	Mm.PT.58.10419414	
<i>Cspg4</i>	qPCR	Mm.PT.56a.29461721	
<i>Cxcl12</i>	qPCR	Mm.PT.56a.7098583.g	

<i>Fap</i>	qPCR	Mm.PT.56a.31960536
<i>Gapdh</i>	qPCR	Mm.PT.39a.1
<i>Grem1</i>	qPCR	Mm.PT.53a.31803129
<i>Id2</i>	qPCR	Mm.PT.58.13116812.g
<i>Kitl</i>	qPCR	Mm.PT.56a.6382703
<i>Klf4</i>	qPCR	Mm.PT.56a.10779296
<i>Lepr</i>	qPCR	Mm.PT.56a.33275723
<i>Myod</i>	qPCR	Mm.PT.56a.8193525
<i>Nes</i>	qPCR	Mm.PT.56a.5953887
<i>Pparg</i>	qPCR	Mm.PT.56a.31161924
<i>Runx2</i>	qPCR	Mm.PT.56a.31234632.g
<i>Sox9</i>	qPCR	Mm.PT.56a.42739087
<i>Sp7</i> (<i>Osterix</i>)	qPCR	Mm.PT.56a.10898265
<i>Wnt4</i>	qPCR	Mm.PT.56a.8723468
<i>GAPDH</i> (human)	qPCR	Hs.PT.39a.22214836
<i>GREM1</i> (human)	qPCR	Hs.PT.53a.26917089.g
<i>Acta2-RFP</i> and <i>R26-mT/mG</i>	Phenotyping	Carriers were detected by red fluorescence of the tail

Table 1B: Mice used in our study

Mouse line used	Reference	Received from	Description
<i>Grem1-creER^T</i>	Published here	Generated in the Wang lab.	A BAC transgenic in which the regulatory elements of <i>Grem1</i> drive expression of a tamoxifen inducible Cre recombinase. <i>Grem1</i> expressing cells express Cre recombinase that can, following binding of tamoxifen, translocate to the nucleus to recombine and thus activate fluorescent reporters or the diphtheria toxin subunit A lines described below.
<i>Grem1-LacZ</i>	Khokha et al, 2003	investigator	A LacZ reporter is knocked in to the endogenous <i>Grem1</i> locus. Cells that express <i>Grem1</i> can be detected by LacZ activity.

<i>Nes-creER^T</i>	Dranovsky et al., 2011	investigator	A transgenic line in which a short regulatory sequence of <i>Nes</i> drives expression of a tamoxifen inducible Cre recombinase. Following administration of tamoxifen, <i>Nes</i> expressing cells express Cre recombinase that can translocate to the nucleus to recombine and thus activate fluorescent reporters. In this line a 5.3kb region drives the CreERT2 upstream of the transcriptional start site of the Nestin gene. This fragment is fused to the CreERT2 followed by a polyA tail and then a 653bp region of the 2nd intron (corresponding to base pair positions 2880—>3533 downstream of the start site).
<i>Nes-GFP</i>	Mignone et al., 2004	investigator	A transgenic line in which a short regulatory sequence of <i>Nes</i> drives GFP expression.
<i>2.3ColGFP</i>	Kalajzic et al., 2002	JAX stock no. 13134	A transgenic line in which a short (2.3kb) regulatory sequence of rat <i>Col1a1</i> drives GFP expression. This line is used to identify committed osteoblasts in mice.
<i>Acta2-RFP</i>	Magness et al., 2004	investigator	A transgenic line in which a short regulatory sequence of <i>Acta2</i> drives RFP expression.
<i>R26-LSL-TdTomato</i>	Madisen et al., 2010	JAX stock no. 7909	A <i>cre</i> recombinase activated red fluorescent reporter knocked into the <i>Rosa26</i> locus.

<i>R26-LSL- ZsGreen</i>	Madisen et al., 2010	JAX stock no. 7906	A <i>cre</i> recombinase activated green fluorescent reporter knocked into the Rosa26 locus.
<i>R26-LSL-mT/mG</i>	Muzumdar et al., 2007	JAX stock no. 7676	A <i>cre</i> recombinase activated green fluorescent reporter knocked into the Rosa26 locus. Prior to recombination all cells express a red fluorescent protein, which allows easier appreciation of tissue architecture on fluorescent microscopy.
<i>R26-LSL-Confetti</i>	Snippert et al., 2010	JAX stock no. 17492	A <i>cre</i> recombinase activated fluorescent reporter knocked into the Rosa26 locus. The value of this reporter is that one of 4 fluorescent tags is expressed randomly following recombination, either, YFP, RFP, mCFP or nGFP. This allows clones to be mapped <i>in vivo</i> .
<i>R26-LSL-DTA</i>	Voehringer et al., 2008	JAX stock no. 9669	A <i>cre</i> recombinase activated diphtheria toxin subunit A expressing construct knocked into the Rosa26 locus. This allowed cell specific programmed cell death.

Table 1C: Other mice previously reported as marking mesenchymal stem/progenitor cells, not included in our study

Mouse line used	Reported in	Description
<i>Lepr-cre</i>	Ding et al, 2012; Zhou et al 2014, Mizoguchi et al, 2014.	<i>Lepr-cre</i> is a knock-in, constitutive <i>Cre</i> line that primarily identifies perisinusoidal mesenchymal cells in the bone marrow. Across 3 papers the perisinusoidal cells it marks have been shown to not generate bone or cartilage in early post-natal life, but that in later life beginning around 2 months, <i>Lepr</i> ⁺ perisinusoidal cells begin to differentiate into bone and adipocytes and, in fracture callus, chondrocytes.
<i>Nes-Cre</i>	Mendez-Ferrer, et al, 2010; first published in Tronche et al, 1999.	Lineage tracing of bone and cartilage has been reported from this line, albeit that the recombination may not perfectly mimic the perisinusoidal distribution of <i>Nes-GFP</i> .
<i>Nes-CreERT</i>	Mendez-Ferrer, et al, 2010; first published in Balordi & Fishell, 2007.	Lineage tracing of bone and cartilage has been reported from this line, albeit that the recombination may again not perfectly mimic the perisinusoidal distribution of <i>Nes-GFP</i> .
<i>Osx-Cre</i>	Mizoguchi et al, 2014. Maes et al, 2010, Park, et al 2012.	<i>Osterix (Sp7)</i> is a broad mesenchymal marker, but in adulthood is recognized as an early, but non-self-renewing, osteolineage marker. However, <i>Osx</i> is expressed in cell populations of varying potential throughout development and it was recently shown that perinatal <i>Osx</i> expressing cells (P5) are long-lived with bone and stromal potential.
<i>Ng2-CreERT</i>	Kunisaki et al, 2013; Feng, et al, 2011.	In the adult murine bone marrow <i>Ng2-CreERT</i> ⁺ identifies periaarteriolar cells, important for the HSC niche. In the tooth <i>Ng2</i> ⁺ cells are mesenchymal stem/progenitor cells generating odontoblasts.
<i>Acta2-CreER</i>	Grcevic et al, 2012.	<i>Acta2</i> expressing cells act as <i>in vitro</i> and <i>in vivo</i> mesenchymal stem/progenitor cells. <i>Acta2</i> is also expressed in smooth muscle and thus is probably expressed in both differentiated and less differentiated mesenchymal populations.
<i>Prx1-Cre</i>	Logan et al, 2002.	<i>Prx1</i> is expressed in early limb bud mesoderm and as a constitutive <i>Cre</i> will thus trace all lineages derived from limb bud mesoderm including bone, stroma and cartilage. It has been used in studies requiring broad mesodermal tracing and conditional knockouts.

Table 1D: Antibodies

Primary antibody	Clone	Purpose	Manufacturer	Catalog number	Conjugated/Biotinylated	Dilution
Sca-1	D7	FC	BioLegend	108116	Alexa Fluor ® 488	0.388888889
CD105	MJ7/18	FC	BioLegend	120405	Alexa Fluor ® 488	0.388888889
CD31	MEC13.3	FC	BioLegend	102509	APC	0.388888889
CD45	30-F11	FC	BioLegend	103111	APC	0.388888889
TER-119	TER-119	FC	BioLegend	116211	APC	0.388888889
Sca-1	D7	FC	BioLegend	108125	APC/Cy7	0.388888889
CD140a	APA5	FC	BioLegend	135905	PE	0.388888889
CD105	MJ7/18	FC/IF	BioLegend	120404	BIOTINYLATED	0.388888889
CD105	MJ7/18	FC	BioLegend	120410	PE/Cy7	0.388888889
CD140a	APA5	FC	BioLegend	135910	BIOTINYLATED	0.388888889
GFP	Polyclonal	IF	Invitrogen	A-11122	None (rabbit anti-GFP)	0.180555556
CD31	MEC 13.3	IF	BD Biosciences	550274	None (LEW rat host anti-mouse CD31)	0.180555556
Sox9	Polyclonal	IF	Millipore	ABE571	None	0.180555556
Osteocalcin	Polyclonal	IF	Takara	M173	None	0.180555556
Perilipin	D1D8	IF	Cell Signalling	9349	None	0.180555556
pSmad1,5	31H14L11	FC	Invitrogen	700047	None	0.388888889
Ng2	polyclonal	IF	Millipore	AB5320	None	0.180555556
TdTomato	Polyclonal	TEM	Clontech	632496	None (rabbit anti-dsRed worked for TdTomato)	0.388888889

Table 1E: Bmp2 pathway Genes, Grem1+VsGrem1- fdr<.05

Symbol	Description	Log₂ FC
Acvr1	activin A receptor, type 1	6.94
Bmp2	bone morphogenetic protein 2	5.79
Bmp5	bone morphogenetic protein 5	4.36
Bmp6	bone morphogenetic protein 6	2.18
Id2	inhibitor of DNA binding 2	2.56

Table 1F: Grem1+ vs Grem1 - Significant KEGG Pathways

Rank	Name	ID	pSize	onArray
1	ECM-receptor interaction	4512	87	85
2	PI3K-Akt signaling pathway	4151	356	333
3	Focal adhesion	4510	205	203
4	Fc gamma R-mediated phagocytosis	4666	89	87
5	Natural killer cell mediated cytotoxicity	4650	125	112
6	Osteoclast differentiation	4380	127	118
7	Amoebiasis	5146	120	113
8	Proteoglycans in cancer	5205	230	222
9	Cytokine-cytokine receptor interaction	4060	266	227
10	B cell receptor signaling pathway	4662	78	77
11	Regulation of actin cytoskeleton	4810	217	210
12	Aldosterone-regulated sodium reabsorption	4960	40	40
13	Fc epsilon RI signaling pathway	4664	71	70
14	MAPK signaling pathway	4010	259	253
15	HIF-1 signaling pathway	4066	113	104
16	VEGF signaling pathway	4370	66	66
17	Leishmaniasis	5140	66	66
18	Jak-STAT signaling pathway	4630	156	136
19	Transcriptional misregulation in cancer	5202	181	164
20	Axon guidance	4360	133	133
21	Phosphatidylinositol signaling system	4070	81	79
22	Long-term depression	4730	61	59
23	Tuberculosis	5152	179	170
24	Bacterial invasion of epithelial cells	5100	77	76
25	Salivary secretion	4970	77	73
26	Pancreatic secretion	4972	103	99
27	Leukocyte transendothelial migration	4670	121	116
28	Rheumatoid arthritis	5323	84	81
29	Viral myocarditis	5416	94	74
30	Renal cell carcinoma	5211	69	68
31	Staphylococcus aureus infection	5150	52	49
32	Glioma	5214	66	64
33	Hepatitis B	5161	149	140
34	Small cell lung cancer	5222	86	85
35	HTLV-I infection	5166	290	266
36	Salmonella infection	5132	79	77
37	Amphetamine addiction	5031	70	68
38	Viral carcinogenesis	5203	236	191
39	GnRH signaling pathway	4912	89	87

Table 1G: Grem1+ vs Grem1 - Significant Reactome Pathways

Rank	Name	ID	pSize	onArray	NDE	tA
1	Collagen biosynthesis and modifying enzymes	5605856	52	50	20	1.96
2	MPS IIIC - Sanfilippo syndrome C	5605255	110	108	25	-0.98
3	MPS IV - Morquio syndrome B	5605261	110	108	25	-0.96
4	MPS VI - Maroteaux-Lamy syndrome	5605253	110	108	25	-0.96
5	MPS II - Hunter syndrome	5605257	110	108	25	-0.95
6	Glycosaminoglycan metabolism	5605249	110	108	25	-0.96
7	MPS IX - Natowicz syndrome	5605250	110	108	25	-0.95
8	MPS VII - Sly syndrome	5605256	110	108	25	-0.94
9	MPS I - Hurler syndrome	5605258	110	108	25	-0.94
10	MPS IIIB - Sanfilippo syndrome B	5605252	110	108	25	-0.94
11	MPS IIIA - Sanfilippo syndrome A	5605260	110	108	25	-0.94
12	MPS IIID - Sanfilippo syndrome D	5605259	110	108	25	-0.93
13	MPS IV - Morquio syndrome A	5605254	110	108	25	-0.95
14	Fcgamma receptor (FCGR) dependent phagocytosis	5605170	72	71	17	-0.53
15	Cell surface interactions at the vascular wall	5605041	83	81	17	1.45
16	Collagen degradation	5605823	31	31	10	1.47
17	Integrin cell surface interactions	5605038	60	58	14	1.00
18	Assembly of collagen fibrils and other multimeric structures	5605828	27	27	9	-0.91
19	Degradation of the extracellular matrix	5605824	94	87	16	-1.05
20	Signaling by Rho GTPases	5605351	100	97	15	1.77
21	GPVI-mediated activation cascade	5605042	26	26	8	0.21
22	Response to elevated platelet cytosolic Ca ²⁺	5605037	86	81	14	-0.17
23	Syndecan interactions	5605962	18	18	6	0.37
24	Elastic fibre formation	5605850	36	36	7	-1.32
25	Signaling by Hippo	5605905	20	20	6	0.29
26	Activation of Matrix Metalloproteinases	5605848	45	39	8	0.93
27	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	5605383	51	43	7	1.66
28	Regulation of lipid metabolism by Peroxisome proliferator-activated receptor alpha (PPARalpha)	5605592	73	73	11	-1.10

Table 1G, cont.

NDE	tA	pNDE	pPERT	pG	pG FDR	Status
26	4.91	4.E-12	0.00	1.E-08	2.E-06	Activated
48	2.53	3.E-08	0.02	3.E-07	2.E-05	Activated
49	2.36	6.E-17	0.02	4.E-07	2.E-05	Activated
27	1.53	1.E-12	0.12	2.E-06	7.E-05	Not Applicable
21	2.12	4.E-06	0.04	2.E-06	7.E-05	Activated
23	0.94	7.E-07	0.34	5.E-06	1.E-04	Not Applicable
25	0.65	2.E-08	0.52	8.E-06	2.E-04	Not Applicable
36	0.44	9.E-08	0.66	1.E-05	2.E-04	Not Applicable
32	-1.70	1.E-05	0.08	1.E-05	2.E-04	Not Applicable
18	0.13	7.E-07	0.89	1.E-05	2.E-04	Not Applicable
34	-0.10	2.E-07	0.92	1.E-05	2.E-04	Not Applicable
11	-0.46	2.E-05	0.67	2.E-04	2.E-03	Not Applicable
14	1.00	8.E-05	0.30	3.E-04	3.E-03	Not Applicable
33	0.07	4.E-05	0.94	4.E-04	4.E-03	Not Applicable
16	1.71	6.E-04	0.07	5.E-04	4.E-03	Not Applicable
13	-1.06	2.E-04	0.28	5.E-04	4.E-03	Not Applicable
13	0.88	2.E-04	0.36	6.E-04	5.E-03	Not Applicable
20	-0.76	2.E-04	0.46	1.E-03	9.E-03	Not Applicable
22	-0.47	5.E-04	0.34	2.E-03	0.01	Not Applicable
19	-0.83	5.E-04	0.40	2.E-03	0.01	Not Applicable
13	-0.73	1.E-03	0.30	3.E-03	0.02	Not Applicable
9	2.11	9.E-03	0.04	3.E-03	0.02	Activated
22	0.67	8.E-04	0.49	3.E-03	0.02	Not Applicable
13	-0.36	7.E-04	0.72	4.E-03	0.02	Not Applicable
12	-0.68	2.E-03	0.50	6.E-03	0.03	Not Applicable
15	-0.24	1.E-03	0.82	7.E-03	0.04	Not Applicable
16	0.71	2.E-03	0.46	7.E-03	0.04	Not Applicable
11	1.53	1.E-02	0.09	7.E-03	0.04	Not Applicable
12	-0.61	2.E-03	0.54	8.E-03	0.04	Not Applicable
11	-0.88	3.E-03	0.39	8.E-03	0.04	Not Applicable
8	-1.43	9.E-03	0.12	9.E-03	0.04	Not Applicable
10	-1.11	5.E-03	0.22	9.E-03	0.04	Not Applicable
18	-0.62	2.E-03	0.53	1.E-02	0.04	Not Applicable
12	1.19	6.E-03	0.23	1.E-02	0.04	Not Applicable
29	0.41	2.E-03	0.68	1.03E-02	0.040327256	Not Applicable
12	0.44	2.E-03	0.65	1.20E-02	0.045777725	Not Applicable
11	0.49	3.E-03	0.63	1.26E-02	0.0467475	Not Applicable
22	-0.70	3.E-03	0.57	1.41E-02	0.049467655	Not Applicable
12	0.98	7.E-03	0.29	1.41E-02	0.049467655	Not Applicable

pNDE	pPERT	pG	pG FDR	Status
0.E+00	0.05	0.00	7.E-05	Activated
0.E+00	0.32	0.00	7.E-05	Not Applicable
0.E+00	0.33	0.00	7.E-05	Not Applicable
0.E+00	0.34	0.00	7.E-05	Not Applicable
0.E+00	0.34	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.35	0.00	7.E-05	Not Applicable
0.E+00	0.37	0.00	7.E-05	Not Applicable
1.E-06	0.61	0.00	1.E-04	Not Applicable
7.E-06	0.13	0.00	1.E-04	Not Applicable
1.E-05	0.13	0.00	2.E-04	Not Applicable
8.E-06	0.34	0.00	3.E-04	Not Applicable
2.E-05	0.56	0.00	1.E-03	Not Applicable
7.E-05	0.28	0.00	2.E-03	Not Applicable
8.E-04	0.06	0.00	4.E-03	Not Applicable
1.E-04	0.83	0.00	0.01	Not Applicable
4.E-04	0.93	0.00	0.02	Not Applicable
5.E-04	0.74	0.00	0.02	Not Applicable
6.E-03	0.12	0.01	0.03	Not Applicable
1.E-03	0.80	0.01	0.04	Not Applicable
2.E-03	0.36	0.01	0.04	Not Applicable
1.E-02	0.07	0.01	0.04	Not Applicable
5.E-03	0.23	0.01	0.04	Not Applicable

Table 1H: ECM-Receptor Interaction Genes in Grem1+ Vs Grem1- (fdr<0.05)

Symbol	Description	Log₂ FC
Chad	chondroadherin	5.39
Col11a1	collagen, type XI, alpha 1	3.77
Col11a2	collagen, type XI, alpha 2	4.14
Col27a1	collagen, type XXVII, alpha 1	2.88
Col4a2	collagen, type IV, alpha 2	1.49
Col4a5	collagen, type IV, alpha 5	4.27
Col5a1	collagen, type V, alpha 1	3.09
Col5a2	collagen, type V, alpha 2	2.49
Col6a1	collagen, type VI, alpha 1	5.01
Col6a2	collagen, type VI, alpha 2	4.00
Col6a3	collagen, type VI, alpha 3	4.66
Comp	cartilage oligomeric matrix protein	4.00
Dag1	dystroglycan 1	2.35
Hspg2	perlecan (heparan sulfate proteoglycan 2)	2.60
Ibsp	integrin binding sialoprotein	3.26
Itga4	integrin alpha 4	-4.24
Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
Itga6	integrin alpha 6	2.17
Itgav	integrin alpha V	2.81
Itgb3	integrin beta 3	-1.62
Lama4	laminin, alpha 4	1.80
Lamb2	laminin, beta 2	2.50
Npnt	nephronectin	3.91
Sdc1	syndecan 1	2.76
Sdc4	syndecan 4	4.91
Thbs3	thrombospondin 3	4.57

	A	B	C
1	Table 11: PI3K-Akt Signaling Genes in Grem1+ Vs Grem1- (fdr<0.05)		
2	Symbol	Description	Log₂ FC
3	Ccnd1	cyclin D1	4.35
4	Ccnd3	cyclin D3	-2.04
5	Chad	chondroadherin	5.39
6	Col11a1	collagen, type XI, alpha 1	3.77
7	Col11a2	collagen, type XI, alpha 2	4.14
8	Col27a1	collagen, type XXVII, alpha 1	2.88
9	Col4a2	collagen, type IV, alpha 2	1.49
10	Col4a5	collagen, type IV, alpha 5	4.27
11	Col5a1	collagen, type V, alpha 1	3.09
12	Col5a2	collagen, type V, alpha 2	2.49
13	Col6a1	collagen, type VI, alpha 1	5.01
14	Col6a2	collagen, type VI, alpha 2	4.00
15	Col6a3	collagen, type VI, alpha 3	4.66
16	Comp	cartilage oligomeric matrix protein	4.00
17	Creb3l2	cAMP responsive element binding protein 3-like 2	4.85
18	Csf1r	colony stimulating factor 1 receptor	-1.73
19	Fgf2	fibroblast growth factor 2	5.70
20	Fgfr1	fibroblast growth factor receptor 1	2.60
21	Fgfr2	fibroblast growth factor receptor 2	5.65
22	Fgfr3	fibroblast growth factor receptor 3	3.86
23	Ibsp	integrin binding sialoprotein	3.26
24	Igf1	insulin-like growth factor 1	4.11
25	Itga4	integrin alpha 4	-4.24
26	Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
27	Itga6	integrin alpha 6	2.17
28	Itgav	integrin alpha V	2.81
29	Itgb3	integrin beta 3	-1.62
30	Lama4	laminin, alpha 4	1.80
31	Lamb2	laminin, beta 2	2.50
32	Lpar1	lysophosphatidic acid receptor 1	3.03
33	Lpar4	lysophosphatidic acid receptor 4	4.46
34	Lpar6	lysophosphatidic acid receptor 6	-3.83
35	Mapk1	mitogen-activated protein kinase 1	-3.32
36	Myb	myeloblastosis oncogene	-5.06
37	Ngf	nerve growth factor	5.93
38	Osmr	oncostatin M receptor	5.40
39	Pdgfa	platelet derived growth factor, alpha	1.50
40	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	-2.58
41	Pten	phosphatase and tensin homolog	-3.97
42	Ptk2	PTK2 protein tyrosine kinase 2	2.83
43	Rheb	Ras homolog enriched in brain	1.33
44	Sgk1	serum/glucocorticoid regulated kinase 1	2.45
45	Sos2	son of sevenless homolog 2 (Drosophila)	-2.70
46	Syk	spleen tyrosine kinase	-4.15
47	Thbs3	thrombospondin 3	4.57
48	Tlr4	toll-like receptor 4	-2.88
49	Vegfa	vascular endothelial growth factor A	1.87
50	Ywha	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	1.77

Table 1J: Focal Adhesion Genes in Grem1+ Vs Grem1- (fdr<0.05)

Symbol	Description	Log₂ FC
Actn4	actinin alpha 4	1.47
Capn2	calpain 2	3.45
Cav1	caveolin 1, caveolae protein	3.07
Cav2	caveolin 2	3.08
Ccnd1	cyclin D1	4.35
Ccnd3	cyclin D3	-2.04
Chad	chondroadherin	5.39
Col11a1	collagen, type XI, alpha 1	3.77
Col11a2	collagen, type XI, alpha 2	4.14
Col27a1	collagen, type XXVII, alpha 1	2.88
Col4a2	collagen, type IV, alpha 2	1.49
Col4a5	collagen, type IV, alpha 5	4.27
Col5a1	collagen, type V, alpha 1	3.09
Col5a2	collagen, type V, alpha 2	2.49
Col6a1	collagen, type VI, alpha 1	5.01
Col6a2	collagen, type VI, alpha 2	4.00
Col6a3	collagen, type VI, alpha 3	4.66
Comp	cartilage oligomeric matrix protein	4.00
Flnb	filamin, beta	2.89
Flnc	filamin C, gamma	3.35
Ibsp	integrin binding sialoprotein	3.26
Igf1	insulin-like growth factor 1	4.11
Itga4	integrin alpha 4	-4.24
Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
Itga6	integrin alpha 6	2.17
Itgav	integrin alpha V	2.81
Itgb3	integrin beta 3	-1.62
Jun	jun proto-oncogene	1.98
Lama4	laminin, alpha 4	1.80
Lamb2	laminin, beta 2	2.50
Mapk1	mitogen-activated protein kinase 1	-3.32
Mylk	myosin, light polypeptide kinase	2.14
Pak3	p21 protein (Cdc42/Rac)-activated kinase 3	5.49
Parvg	parvin, gamma	-3.07
Pdgfa	platelet derived growth factor, alpha	1.50
Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	-2.58
Prkcb	protein kinase C, beta	-3.85
Prkcg	protein kinase C, gamma	2.75

Pten	phosphatase and tensin homolog	-3.97
Ptk2	PTK2 protein tyrosine kinase 2	2.83
Rac2	RAS-related C3 botulinum substrate 2	-3.03
Sos2	son of sevenless homolog 2 (Drosophila)	-2.70
Thbs3	thrombospondin 3	4.57
Vasp	vasodilator-stimulated phosphoprotein	-1.39
Vav1	vav 1 oncogene	-4.46
Vav2	vav 2 oncogene	2.38
Vav3	vav 3 oncogene	-2.01
Vegfa	vascular endothelial growth factor A	1.87
Xiap	X-linked inhibitor of apoptosis	-2.45

Table 1K: Osteoblast differentiation GO:0001649

Symbol	probeid	logFC	P.Value	fdr
Shox2	1438042_at	7.139693094	1.12E-05	0.024717328
Fgfr2	1433489_s_at	5.652317879	1.51E-05	0.024717328
Bmp2	1423635_at	5.787265063	5.84E-05	0.024717328
Col9a1	1421381_a_at	5.143849185	7.20E-05	0.024717328
Sox9	1424950_at	6.90133905	0.000110199	0.024717328
Comp	1419527_at	3.999527882	0.00013709	0.025235259
Papss2	1421987_at	5.85768973	0.000137253	0.025235259
Col10a1	1422253_at	4.649700769	0.000150964	0.025235259
Matn1	1418477_at	5.63933284	0.000191855	0.026424026
Impad1	1437290_at	4.000095671	0.000198075	0.02656323
Ltbp3	1437833_at	2.966172299	0.000217433	0.027040111
Sp7	1418425_at	3.103511735	0.000261664	0.028624838
Ddr2	1422738_at	3.897227684	0.000269907	0.028738428
Ankrd11	1458452_at	-1.749643873	0.000427813	0.03083902
Bmp6	1450759_at	2.177660599	0.000527626	0.032374884
Thbs3	1416623_at	4.56764372	0.000569659	0.033236981
Ptprc	1440165_at	-5.673465126	0.000645357	0.03437141
Igf1	1419519_at	4.105829219	0.000766857	0.03552034
Mef2c	1451506_at	1.562297202	0.000772609	0.03552034
Gli3	1456067_at	2.454729863	0.000813139	0.036059697
Insig1	1454671_at	2.083603859	0.000857009	0.036461333
Csgalnact1	1452365_at	6.102113169	0.000870032	0.036461333
Asxl2	1460597_at	-2.69793666	0.000925415	0.036876699
Hspg2	1418670_s_at	2.601492005	0.000988966	0.037703609
Dlx5	1449863_a_at	3.758824499	0.001062068	0.039185564
Sparc	1416589_at	1.604128392	0.001134471	0.040211493
Slc38a10	1427295_at	1.836342464	0.001251799	0.041425472
Has2	1449169_at	4.992358344	0.001262338	0.041642764
Fgfr3	1421841_at	3.862610571	0.001486791	0.044252681
Npr2	1427191_at	3.46860457	0.00168539	0.046605008
Sulf2	1442408_at	1.6036124	0.001818939	0.048072123
Serpinh1	1450843_a_at	1.296545474	0.00188638	0.048519059
Sema4d	1420824_at	-1.802904281	0.001947949	0.04888768
Phospho2	1425190_a_at	-2.814035598	0.001978658	0.049100382
Alpl	1423611_at	2.963453942	0.003152191	0.059797918
Cadm1	1417376_a_at	4.105907278	0.003749737	0.06413052
Smad1	1448208_at	3.171019581	0.003939544	0.065217381
Fat4	1459749_s_at	3.238993925	0.004025324	0.065970771
Runx2	1424704_at	2.887812904	0.005507695	0.0751405

Rarg	1419415_a_at	1.550286712	0.005801297	0.076976842
Insig2	1417980_a_at	2.44324681	0.005844576	0.077271793
Sik3	1460439_at	-1.085764805	0.007557193	0.087776707
Cyp26b1	1460011_at	2.414945578	0.007803403	0.089217497
Glg1	1460554_s_at	1.08231524	0.009548025	0.098119909
Pex7	1418988_at	-1.511451194	0.010079617	0.101400796
Gnas	1450186_s_at	1.15793611	0.01017669	0.101946203
Smad5	1433641_at	1.326053173	0.011763418	0.109476522
Ostc	1449139_at	1.477735339	0.013125314	0.116439497
Plxnb1	1435254_at	2.942409912	0.013429212	0.117743176
Bbx	1425835_a_at	2.446951157	0.014483662	0.122648828
Twist1	1418733_at	1.382611779	0.015769561	0.1278689
Nab1	1438819_at	-2.921685423	0.01616797	0.129413797
Asxl1	1458380_at	-1.336329518	0.020033869	0.144567603
Osr2	1426155_a_at	0.502214387	0.024820681	0.160932229
Setdb1	1451833_a_at	-0.705544003	0.026029982	0.164930909
Pthlh	1422324_a_at	2.22023888	0.027653676	0.170616752
Ift80	1427568_a_at	2.136527636	0.035499982	0.194259243
Col2a1	1450567_a_at	0.516328675	0.036987644	0.198758455
Cited2	1452207_at	0.777201054	0.038182	0.202260558
Pax1	1449359_at	3.129371792	0.039117548	0.205001226
Ski	1426373_at	1.208235773	0.039744837	0.206769456
Rhoa	1437628_s_at	-0.461425196	0.044691344	0.220310888
Bnc2	1438861_at	4.234307253	0.048503083	0.23058618
Whsc1	1435136_at	-1.003323897	0.060212775	0.258609312
Inpp1	1460394_a_at	0.567599274	0.068322178	0.276571991
Mcph1	1439115_at	-1.231750154	0.074267025	0.289484996
Fgf18	1449545_at	1.956305626	0.080130689	0.300802975
Hoxa11	1420414_at	0.511171629	0.134279	0.394218529
Ctc1	1423656_x_at	-0.345446644	0.137792974	0.400270575
Sh3pxd2b	1442919_at	0.783611603	0.137852296	0.40031788
Evc	1448876_at	1.720368653	0.140736918	0.404315813
Lrp6	1451022_at	1.847234643	0.145204659	0.410548216
Grem1	1425357_a_at	2.037426007	0.154104522	0.422971522
Amer1	1439565_at	-1.2063171	0.160046885	0.430901053
Scx	1456291_x_at	0.275344353	0.169500763	0.442948625
Ptger4	1424208_at	-0.972916857	0.178471738	0.455326048
Ryr1	1427306_at	-0.295073248	0.191481139	0.471358493
Rab23	1454876_at	0.859713003	0.194017485	0.474398912
Trim45	1441412_s_at	0.203427908	0.202761269	0.484027734
Axin2	1436845_at	0.387992131	0.211247475	0.49331913

Lrrc17	1429679_at	0.233010363	0.235628038	0.520602564
Pitx2	1424797_a_at	1.070819666	0.236282353	0.521384139
Carm1	1419743_s_at	0.647225506	0.253119301	0.539097732
Dym	1423736_a_at	-0.915006359	0.271266186	0.55550201
Sulf1	1436319_at	1.195688044	0.274329539	0.558100796
Smad9	1450265_at	0.162215826	0.278961897	0.562412139
Prpsap2	1452062_at	-0.314973589	0.28405301	0.566962065
Msx2	1438351_at	0.352902478	0.299225831	0.581271523
Mef2d	1421388_at	-0.186624593	0.317508841	0.598850338
Nppc	1422790_at	0.160740904	0.318516161	0.599649792
Fam73b	1454621_s_at	0.299423097	0.321171428	0.601935055
Acp5	1431609_a_at	-0.930966065	0.331118878	0.610044038
Fgf4	1420086_x_at	0.182639322	0.356485355	0.632486284
Cbs	1423844_s_at	0.200634176	0.366165065	0.640416125
Wnt1	1425377_at	0.369757817	0.379671843	0.650544352
T	1419304_at	0.16388178	0.390584684	0.659124441
Col1a1	1423669_at	-0.621264052	0.406590229	0.671883603
Hoxb4	1451761_at	-0.127266076	0.462604836	0.716107186
Sp5	1422914_at	-0.117409597	0.495346498	0.738630642
Hoxd11	1450584_at	0.09335222	0.516782714	0.752676029
Bmp4	1422912_at	-0.151574562	0.528444464	0.760720517
Fgf8	1451882_a_at	0.082771762	0.563508165	0.784527913
Rarb	1454906_at	0.132469624	0.580742851	0.795459147
Msx1	1417127_at	-0.07927679	0.6088118	0.812199278
Por	1416933_at	-0.080981309	0.640208478	0.830405871
Thbs1	1460302_at	-0.09408991	0.640329897	0.830419322
Rara	1450180_a_at	-0.145032547	0.656889118	0.839241871
Lrp5	1449299_at	0.120069936	0.667173815	0.845349241
Tfap2a	1421996_at	0.048827656	0.730112475	0.878838306
Lep	1422582_at	-0.05140898	0.743335178	0.886299367
Spns2	1451601_a_at	-0.093090513	0.750770457	0.890055922
Nab2	1417930_at	-0.141428281	0.763601435	0.897204332
Sfrp2	1448201_at	0.267663483	0.765342683	0.898105369
Dchs1	1429163_at	0.034058005	0.851902726	0.938492662
Sbds	1426480_at	-0.065123897	0.882072035	0.950943967
Bglap2	1449880_s_at	0.030899771	0.961461518	0.983599805
Cdx1	1449582_at	-0.001241112	0.992657152	0.997194845
Frem1	1455280_at	0.0010303	0.993945229	0.997823631

Table 1L: Chondrocyte differentiation GO:0002062

Symbol	probeid	logFC	P.Value	globalfdr
Sox5	1452511_at	7.227671317	7.96E-06	0.02471733
Frzb	1416658_at	6.487492378	8.83E-06	0.02471733
Shox2	1438042_at	7.139693094	1.12E-05	0.02471733
Trps1	1438214_at	3.407552042	4.76E-05	0.02471733
Cytl1	1456793_at	6.874597272	5.09E-05	0.02471733
Bmp2	1423635_at	5.787265063	5.84E-05	0.02471733
Col11a2	1423578_at	4.142066889	6.46E-05	0.02471733
Pkdcc	1454838_s_at	7.289629759	6.74E-05	0.02471733
Col9a1	1421381_a_at	5.143849185	7.20E-05	0.02471733
Sox9	1424950_at	6.90133905	0.000110199	0.02471733
Tgfb2	1423250_a_at	5.546499385	0.000122435	0.02520744
Comp	1419527_at	3.999527882	0.00013709	0.02523526
Col10a1	1422253_at	4.649700769	0.000150964	0.02523526
Matn1	1418477_at	5.63933284	0.000191855	0.02642403
Impad1	1437290_at	4.000095671	0.000198075	0.02656323
Sox6	1447655_x_at	3.042240366	0.00020449	0.0267325
Ltbp3	1437833_at	2.966172299	0.000217433	0.02704011
Nfib	1434101_at	2.200370996	0.000300679	0.02873843
Chst11	1450509_at	4.028847262	0.000322352	0.02907679
Creb3l2	1452381_at	4.852931274	0.00046023	0.03115804
Bmp6	1450759_at	2.177660599	0.000527626	0.03237488
Col11a1	1418599_at	2.341265056	0.000531438	0.03247748
Thbs3	1416623_at	4.56764372	0.000569659	0.03323698
Mmp13	1417256_at	4.244523746	0.000617022	0.03397116
Cyr61	1438133_a_at	5.691198456	0.00063681	0.03419138
Mia3	1459984_at	2.370589971	0.000658158	0.03447572
Hif1a	1448183_a_at	1.72827087	0.000744244	0.03532241
Bmp5	1455851_at	4.361119404	0.000754542	0.03535411
Mef2c	1451506_at	1.562297202	0.000772609	0.03552034
Fgf2	1449826_a_at	5.704557594	0.000783732	0.03566748
Gli3	1456067_at	2.454729863	0.000813139	0.0360597
Pth1r	1417092_at	5.144468665	0.000813154	0.0360597
Acan	1449827_at	3.418337262	0.000822404	0.03611613
Fgfr1	1424050_s_at	2.601293712	0.000865071	0.03646133
Csgalnact1	1452365_at	6.102113169	0.000870032	0.03646133
Hspg2	1418670_s_at	2.601492005	0.000988966	0.03770361
Mgp	1448416_at	3.350708397	0.001161888	0.0404339
Snai2	1418673_at	5.037011523	0.001285815	0.04171829
Fgfr3	1421841_at	3.862610571	0.001486791	0.04425268

Maf	1437473_at	3.769803046	0.001536869	0.04489271
Ctgf	1416953_at	2.49942916	0.001724906	0.04701858
Sulf2	1442408_at	1.6036124	0.001818939	0.04807212
Serpinh1	1450843_a_at	1.296545474	0.00188638	0.04851906
Thra	1443952_at	1.825949647	0.00237175	0.05300708
Zbtb16	1419874_x_at	3.803773056	0.002398106	0.05325226
Bmp8a	1449873_at	2.201924918	0.003848633	0.06493723
Smad1	1448208_at	3.171019581	0.003939544	0.06521738
Pkd1	1460210_at	2.908566799	0.00405261	0.06613797
Bmp7	1418910_at	1.926967359	0.004189368	0.06714193
Mex3c	1444701_at	2.842755288	0.004395625	0.06871649
Bmpr1a	1425492_at	1.362007729	0.005169266	0.07349907
Runx2	1424704_at	2.887812904	0.005507695	0.0751405
Rarg	1419415_a_at	1.550286712	0.005801297	0.07697684
Barx2	1421761_a_at	0.856240972	0.006409754	0.08068276
Tgfb1	1445360_at	-1.853388092	0.006592491	0.08152761
Lect1	1460258_at	4.589863169	0.006972883	0.08388477
Ctnnb1	1450008_a_at	1.662619268	0.007200751	0.08544095
Tgfb2	1426397_at	1.866595465	0.007508601	0.08755963
Sik3	1460439_at	-1.085764805	0.007557193	0.08777671
Hoxc4	1422870_at	1.197960754	0.007610561	0.08803383
Otor	1425083_at	4.223579518	0.008769642	0.09435106
Ror2	1457128_at	4.339094191	0.008963	0.09531397
Glg1	1460554_s_at	1.08231524	0.009548025	0.09811991
Tgfb2	1420893_a_at	-1.55359297	0.009766074	0.09956142
Gnas	1450186_s_at	1.15793611	0.01017669	0.1019462
Smad5	1433641_at	1.326053173	0.011763418	0.10947652
Prkca	1427562_a_at	2.106132835	0.013587175	0.11834592
Hes5	1456010_x_at	-2.12314448	0.016556928	0.13093705
Gli2	1459211_at	1.040592945	0.017675299	0.13518291
Mapk14	1426104_at	-1.553226091	0.020975461	0.14796945
Lnp	1453035_at	-2.975528114	0.023920912	0.15807429
Osr2	1426155_a_at	0.502214387	0.024820681	0.16093223
Hmga2	1450781_at	1.095868613	0.02694941	0.16831614
Pthlh	1422324_a_at	2.22023888	0.027653676	0.17061675
Wnt9a	1436978_at	0.87174088	0.027858165	0.17122255
Bmpr1b	1437312_at	1.259734785	0.028010413	0.17173704
Prrx1	1432129_a_at	1.477735949	0.028062403	0.17191557
Foxd1	1418876_at	4.467305245	0.030439858	0.17907112
Ift80	1427568_a_at	2.136527636	0.035499982	0.19425924
Col2a1	1450567_a_at	0.516328675	0.036987644	0.19875846

Thrb	1422202_at	2.480665058	0.043633913	0.21759789
Atp7a	1418774_a_at	-1.673902233	0.044739406	0.22045143
Mapk3	1427060_at	-0.665697033	0.049130252	0.23233967
Wnt5a	1448818_at	2.932630314	0.049273596	0.23264251
Bmp1	1427457_a_at	2.430286862	0.051273483	0.23736193
Rela	1419536_a_at	1.047678744	0.057679732	0.25345212
Hoxa5	1443803_x_at	1.027593295	0.061492348	0.26170129
Esrra	1442864_at	-0.644689343	0.067250466	0.27435072
Fgf18	1449545_at	1.956305626	0.080130689	0.30080298
Hoxa3	1427433_s_at	0.507084998	0.08120338	0.30287153
Zbtb7a	1437255_at	-0.366464305	0.09699201	0.33330316
Runx3	1440275_at	0.967803284	0.097930192	0.33494632
Hoxb3	1427605_at	0.394183877	0.099739178	0.33804288
Edn1	1451924_a_at	-0.298299622	0.101953846	0.34187512
Eif2ak3	1430371_x_at	-1.422549648	0.119792665	0.37163913
Satb2	1425904_at	0.486466036	0.128215418	0.38470549
Bbs1	1437310_at	1.90386954	0.128294835	0.38470549
Hoxa11	1420414_at	0.511171629	0.134279	0.39421853
Bmp8b	1440706_at	0.263087681	0.135557361	0.39655082
Foxd2	1442315_at	0.463202134	0.143844177	0.40876544
Lrp6	1451022_at	1.847234643	0.145204659	0.41054822
Zeb1	1418926_at	1.163547486	0.146603252	0.41268652
Fgf9	1438718_at	-0.396660104	0.159287355	0.42979473
Scx	1456291_x_at	0.275344353	0.169500763	0.44294863
Wnt7a	1458334_at	0.230585657	0.178295657	0.45510852
Mkks	1422627_a_at	0.629498974	0.204234917	0.48574462
Bbs2	1424478_at	1.188472075	0.204515227	0.48587449
Axin2	1436845_at	0.387992131	0.211247475	0.49331913
Arid5a	1451340_at	-0.312811068	0.211944957	0.49438477
Hand1	1417525_at	0.201272013	0.240887009	0.52659659
Carm1	1419743_s_at	0.647225506	0.253119301	0.53909773
Sulf1	1436319_at	1.195688044	0.274329539	0.5581008
Smad9	1450265_at	0.162215826	0.278961897	0.56241214
Mycn	1417155_at	0.397691657	0.289395697	0.57218164
Pitx1	1419514_at	0.170296898	0.291463694	0.57395417
Msx2	1438351_at	0.352902478	0.299225831	0.58127152
Prrx2	1432331_a_at	0.731912166	0.300918486	0.58270253
Wnt7b	1420892_at	0.157152661	0.314547758	0.59632366
Mef2d	1421388_at	-0.186624593	0.317508841	0.59885034
Nppc	1422790_at	0.160740904	0.318516161	0.59964979
Chrdl2	1420539_a_at	0.203324878	0.341475431	0.61922033

Uncx	1419633_at	0.148759176	0.353940851	0.62995605
Fgf4	1420086_x_at	0.182639322	0.356485355	0.63248628
Cbs	1423844_s_at	0.200634176	0.366165065	0.64041613
Cst10	1449447_at	0.197121868	0.368427113	0.6420478
Gdf5	1419139_at	0.129088056	0.371305218	0.6441108
Foxd4	1422318_at	-0.134476132	0.379737298	0.65058051
Foxd3	1422210_at	0.122987443	0.399274562	0.66574299
Col1a1	1423669_at	-0.621264052	0.406590229	0.6718836
Fgf6	1427582_at	0.133060268	0.435640805	0.69508034
Nog	1422300_at	0.153211198	0.443767114	0.70129789
Smad3	1450472_s_at	0.284666876	0.448289325	0.70498612
Fbxw4	1417226_at	0.154450778	0.489472582	0.73441242
Six2	1427436_at	0.117987176	0.493264422	0.73685768
Dlx2	1448877_at	0.179930447	0.501440307	0.74322059
Hoxd11	1450584_at	0.09335222	0.516782714	0.75267603
Bmp4	1422912_at	-0.151574562	0.528444464	0.76072052
Rarb	1454906_at	0.132469624	0.580742851	0.79545915
Msx1	1417127_at	-0.07927679	0.6088118	0.81219928
Pax7	1452510_at	-0.072603424	0.615871162	0.81680712
Por	1416933_at	-0.080981309	0.640208478	0.83040587
Thbs1	1460302_at	-0.09408991	0.640329897	0.83041932
Rara	1450180_a_at	-0.145032547	0.656889118	0.83924187
Osr1	1449350_at	0.066742628	0.657967365	0.83979472
Nkx3-2	1421464_at	0.072593918	0.659884707	0.84131336
Snai1	1448742_at	0.051585999	0.712278255	0.86945623
Hand2	1436041_at	0.051832079	0.719149452	0.87278294
Lep	1422582_at	-0.05140898	0.743335178	0.88629937
Sfrp2	1448201_at	0.267663483	0.765342683	0.89810537
Myf5	1420757_at	0.036879133	0.798434878	0.91361693
Ihh	1450704_at	0.030673687	0.821630534	0.9243161
Efemp1	1427183_at	0.226301546	0.846451572	0.93572931
Hoxd3	1421537_at	-0.019430098	0.903327872	0.96012428
Rspo2	1455893_at	-0.022960144	0.960848418	0.9833494

Table 1M: Adipocyte Differentiation GO:0045444

Symbol	probeid	logFC	P.Value	fdr
Frzb	1416658_at	6.487492378	8.83E-06	0.024717328
Scd1	1415965_at	6.929461289	2.02E-05	0.024717328
Wif1	1425425_a_at	5.61016474	2.97E-05	0.024717328
Wwtr1	1417818_at	4.269668342	4.04E-05	0.024717328
Bmp2	1423635_at	5.787265063	5.84E-05	0.024717328
Medag	1452244_at	2.545095371	0.00012336	0.025207442
Fndc3b	1433833_at	4.141051542	0.00016123	0.025235259
Id2	1435176_a_at	2.559804641	0.000172645	0.025235259
Enpp1	1419276_at	4.181038806	0.000180395	0.025504996
Ccnd1	1448698_at	4.352370653	0.000619696	0.033971157
Selenbp1	1450699_at	-2.072635347	0.000691838	0.034716762
Plcb1	1435043_at	4.482106245	0.000692032	0.034716762
Igf1	1419519_at	4.105829219	0.000766857	0.03552034
4932438A13Rik	1444660_at	-1.721706348	0.000835528	0.036271815
Insig1	1454671_at	2.083603859	0.000857009	0.036461333
Asxl2	1460597_at	-2.69793666	0.000925415	0.036876699
Itga6	1422445_at	2.165019349	0.000951619	0.037127119
Ero1l	1449324_at	-5.241542349	0.001097216	0.039747422
Klf4	1417394_at	2.491431313	0.00117445	0.040597548
Snai2	1418673_at	5.037011523	0.001285815	0.041718287
Lama4	1424807_at	1.798716009	0.001447975	0.043819119
Plac8	1451335_at	-3.934376347	0.001976289	0.049100382
Zbtb16	1419874_x_at	3.803773056	0.002398106	0.053252258
Rgs2	1419248_at	-2.06216508	0.003091707	0.059308851
Id4	1423259_at	3.91112981	0.003232534	0.060264862
Egr2	1427683_at	2.262049947	0.003343364	0.061201783
Gpx1	1460671_at	-1.501819908	0.004191145	0.067141932
1100001G20Rik	1434484_at	-2.836197894	0.004262215	0.067829984
Zfpm2	1449314_at	2.053382872	0.004319297	0.068075477
Mex3c	1444701_at	2.842755288	0.004395625	0.068716491
Lamb3	1417812_a_at	1.656879301	0.004815257	0.071439951
Osbpl8	1437069_at	-1.029145389	0.005234847	0.073806387
Tcf7l2	1429428_at	2.819792433	0.005265046	0.073875105
Fcor	1439834_at	-1.006061729	0.005906145	0.077727492
Nipbl	1442103_at	-1.251092678	0.006463627	0.080954194
Tgfb1	1445360_at	-1.853388092	0.006592491	0.081527606
Psmb8	1422962_a_at	-2.805817714	0.006746438	0.082498168
Creb1	1428755_at	-2.709135357	0.00842715	0.092353841
Bnip3	1422470_at	4.08594527	0.009517431	0.097978919

Creb5	1457222_at	2.782121921	0.011615045	0.108908555
Akt1	1425711_a_at	0.643312065	0.013226622	0.116886603
Pex11a	1419365_at	3.114987589	0.013427369	0.117743176
Wnt5b	1422602_a_at	1.821566134	0.015112995	0.124997466
Adrb2	1437302_at	-1.425485021	0.016312374	0.13005204
Osbp11	1436027_at	-1.513347684	0.016442532	0.130444086
Bbs12	1447275_at	2.085258954	0.016764282	0.131584735
Dact1	1417937_at	2.668260054	0.018729084	0.139389505
Asxl1	1458380_at	-1.336329518	0.020033869	0.144567603
Arl4a	1425411_at	1.291469422	0.020514069	0.146485916
Sox8	1435438_at	3.11014067	0.022232322	0.152924449
Zc3h12a	1443993_at	-1.394639385	0.023186221	0.156129026
Zfp385a	1418865_at	0.542010075	0.023687294	0.157485763
Hmga2	1450781_at	1.095868613	0.02694941	0.168316138
Alms1	1456950_at	-0.917436423	0.026999562	0.168445562
Tgfb1i1	1418136_at	2.245342116	0.03175692	0.182967407
Jdp2	1450350_a_at	-1.716067966	0.038047468	0.201807159
Crebbp	1459804_at	-1.327455321	0.039460203	0.20599124
Lrg1	1417290_at	-1.396551363	0.045891248	0.223712254
Wnt5a	1448818_at	2.932630314	0.049273596	0.232642512
Adipoq	1422651_at	-0.967741253	0.04950593	0.233187147
Sirt1	1418640_at	0.664587861	0.049795512	0.233721239
Jag1	1434070_at	1.602428888	0.050991735	0.236724473
Arid5b	1458238_at	-0.685662171	0.051489457	0.237932994
Ptgs2	1417263_at	1.371927346	0.052627103	0.241065913
Gata2	1450333_a_at	-0.420057378	0.056717396	0.251052809
Med1	1450402_at	-1.571906461	0.059570019	0.257266033
Dlk2	1420807_a_at	0.373678953	0.068401715	0.276729974
Axin1	1426966_at	1.169289	0.069027484	0.27796505
Ppard	1425703_at	0.434121886	0.076751272	0.294402282
Retn	1449182_at	-0.387213077	0.081515149	0.303534902
Cebpa	1418982_at	-0.634142288	0.082465927	0.305385975
Tbl1x	1455042_at	-0.543952328	0.085276819	0.31094428
Cebpb	1418901_at	-0.972281866	0.085444205	0.311252652
Cby1	1451305_at	0.673131945	0.087651962	0.315548462
Mb	1451203_at	-0.51130744	0.088606749	0.317238289
Ncor2	1451841_a_at	0.340384564	0.090456528	0.320981893
Gsk3b	1439931_at	0.834614429	0.090614747	0.32122473
Crebl2	1442738_at	-0.635267227	0.107915586	0.351948864
Aldh6a1	1448104_at	2.581164414	0.108694563	0.353034242
Eif2ak3	1430371_x_at	-1.422549648	0.119792665	0.371639131

Fabp4	1417023_a_at	-1.680412884	0.121138	0.373696644
Ccdc85b	1435589_at	0.279290591	0.122081875	0.375133492
Rarres2	1425091_at	0.316286623	0.127825163	0.384259327
Socs1	1450446_a_at	0.28564968	0.129177821	0.386022436
Sh3pxd2b	1442919_at	0.783611603	0.137852296	0.40031788
Fam57b	1454209_at	0.247150807	0.143889355	0.408784179
Taf8	1416450_at	0.340135345	0.145111022	0.410548216
Lrp6	1451022_at	1.847234643	0.145204659	0.410548216
Runx1t1	1448785_at	0.447542765	0.1596707	0.430417084
Trib2	1426641_at	0.891332721	0.183420649	0.461761357
Ankrd26	1436071_at	0.362057222	0.206617022	0.488193332
Aamdc	1451381_at	1.068486032	0.218997978	0.501958012
Trib3	1426065_a_at	0.295148999	0.233681553	0.51862483
Lpin1	1426516_a_at	0.583917646	0.24135771	0.526996505
Zfpm1	1451046_at	-0.189108731	0.250389917	0.536494732
Noc3l	1437500_at	0.783741682	0.250779312	0.536893509
Carm1	1419743_s_at	0.647225506	0.253119301	0.539097732
Ctbp2	1422887_a_at	-0.404782759	0.270763331	0.554900576
Slc2a4	1415959_at	0.176147069	0.27374878	0.557749536
Msx2	1438351_at	0.352902478	0.299225831	0.581271523
Ctbp1	1415702_a_at	0.63070087	0.307078777	0.58884036
Bscl2	1420632_a_at	0.208707574	0.319770256	0.600814793
Socs7	1420766_at	0.745400207	0.332937222	0.61166653
Prdm16	1429309_at	0.142308012	0.340168904	0.618323642
Sfrp1	1428136_at	0.163111293	0.344049035	0.62158781
Mrap	1451371_at	-0.159923525	0.354449217	0.630342999
Mettl8	1451141_at	0.661628871	0.377374795	0.648726201
Wnt1	1425377_at	0.369757817	0.379671843	0.650544352
Gm6484	1427422_at	0.125398475	0.407923447	0.673018565
Adrb3	1421555_at	-0.101387422	0.481093024	0.728724651
Aloxe3	1449237_at	0.111671705	0.490075015	0.734658995
Sod2	1454976_at	-0.464238608	0.498589239	0.741120235
Sh2b2	1450718_at	0.337876551	0.500932827	0.742889702
Nudt7	1430896_s_at	-0.500929104	0.521799273	0.756028945
Hes1	1418102_at	0.121585835	0.55711349	0.780249527
Gpr116	1440225_at	-0.647155618	0.579033461	0.794203154
Dkk1	1417787_at	0.070573306	0.609619995	0.812529315
Uchl1	1448260_at	0.072150504	0.6101289	0.812826358
Cebpd	1456605_at	0.188950666	0.638667475	0.829432786
Gata3	1448886_at	-0.083381986	0.65162031	0.836542813
Pparg	1420715_a_at	-0.067019888	0.664841034	0.844180446

Fndc5	1435115_at	0.099988573	0.664919216	0.844180446
Lrp5	1449299_at	0.120069936	0.667173815	0.845349241
Adig	1424729_at	0.059571714	0.676763232	0.850717398
Mmp11	1417234_at	0.084863835	0.717623641	0.872175047
Lep	1422582_at	-0.05140898	0.743335178	0.886299367
Sfrp2	1448201_at	0.267663483	0.765342683	0.898105369
Wnt3a	1422093_at	-0.038794612	0.81145358	0.919832305
Adrb1	1423420_at	-0.021770889	0.873212928	0.947461132
Wnt10b	1426091_a_at	0.026764532	0.926524869	0.96924821
Fgf10	1420690_at	-0.003608569	0.986998638	0.99442913

	A	B	C	D
1	Table 1N. Cellular differentiation GO categories represented at different stringencies in Grem1+ Grem1-			
2	Stringency and GO Category	Total Genes in Pathway	Number of Significant Genes in GO category significantly up in at stringency	Fraction of Significant Genes in GO category significantly up in at stringency
3				
4	fdr<0.025			
5	Osteoblast differentiation; GO:0001649	118	5	0.04
6	Chondrocyte Differentiation; GO:000164	154	10	0.06
7	Adipocyte Differentiation; GO:0045444	131	5	0.04
8				
9	fdr<0.05			
10	Osteoblast differentiation; GO:0001649	118	29	0.25
11	Chondrocyte Differentiation; GO:000164	154	43	0.28
12	Adipocyte Differentiation; GO:0045444	131	17	0.13
13				
14	fdr<0.1			
15	Osteoblast differentiation; GO:0001649	118	38	0.32
16	Chondrocyte Differentiation; GO:000164	154	61	0.40
17	Adipocyte Differentiation; GO:0045444	131	24	0.18

Table 2. Primer sequences

Gene	Purpose	Sequence (5'-3')/Sequence name (IDT descriptor)
F-Grem1 recombineering primer	Recombineering	GATTTTACTAAATAACTTTCTTATTGTCTG TGTCCCCCTCTCTTTGTCCTTTGTCTAGAAT GTCCAATTTACTGACCGTACA (SEQ ID NO.: 1)
R-Grem1 recombineering primer	Recombineering	GTTGGCAGTAGGGTCCCCAGGAGGAGAAGC AACGCTCCCACAGTGTATGCGGTGCGATTA TTATGTACCTGACTGATGAAGTT (SEQ ID NO.: 2)
F- Grem1-creERT	Genotyping	CTG TGT CGA ATT ACT CAG TTT GAT G (SEQ ID NO.:3)
R- Grem1-creERT	Genotyping	AAT GTT GCT GGA TAG TTT TTA CTG C (SEQ ID NO.: 4)
F- Grem1-LacZ	Genotyping	ATCCTCTGCATGGTCAGGTC (SEQ ID NO.: 5)
R- Grem1-LacZ	Genotyping	CGTGGCCTGATTCATTCC (SEQ ID NO.: 6)
F- R26-TdTomato or ZsGreen (the WPRE)	Genotyping	ACT GTG TTT GCT GAC GCA AC (SEQ ID NO.: 7)
R- R26-TdTomato or ZsGreen (the WPRE)	Genotyping	CAA CAC CAC GGA ATT GTC AG (SEQ ID NO.: 8)
F- Nes-GFP or 2.3ColGFP or R26-Confetti (the GFP)	Genotyping	GAG CTG AAG GGC ATC GAC TTC AAG (SEQ ID NO.: 9)
R- Nes-GFP or 2.3ColGFP or R26-Confetti (the GFP)	Genotyping	GGA CTG GGT GCT CAG GTA GTG G (SEQ ID NO.: 10)
F- Nes-CreERT	Genotyping	GCG GCA TGG TGC AAG TTG AAT (SEQ ID NO.:11)
R- Nes-CreERT	Genotyping	CGT TCA CCG GCA TCA ACG TTT (SEQ ID NO.: 12)
F- R26-iDTA	Genotyping	ACC TGG TTA TGT AGA TTC CAT TCA A (SEQ ID NO.: 13)
R- R26-iDTA	Genotyping	CAG AAG TAA GGT TCC TTC ACA AAG A (SEQ ID NO.: 14)
<i>Acan</i>	qPCR	Mm.PT.56a.10174685
<i>Angpt2</i>	qPCR	Mm.PT.56a.29139310
<i>Bmp2</i>	qPCR	Mm.PT.58.10419414
<i>Cspg4</i>	qPCR	Mm.PT.56a.29461721
<i>Cxcl12</i>	qPCR	Mm.PT.56a.7098583.g
<i>Fap</i>	qPCR	Mm.PT.56a.31960536
<i>Gapdh</i>	qPCR	Mm.PT.39a.1
<i>Grem1</i>	qPCR	Mm.PT.53a.31803129
<i>Id2</i>	qPCR	Mm.PT.58.13116812.g

<i>Kitl</i>	qPCR	Mm.PT.56a.6382703
<i>Klf4</i>	qPCR	Mm.PT.56a.10779296
<i>Lepr</i>	qPCR	Mm.PT.56a.33275723
<i>Myod</i>	qPCR	Mm.PT.56a.8193525
<i>Nes</i>	qPCR	Mm.PT.56a.5953887
<i>Pparg</i>	qPCR	Mm.PT.56a.31161924
<i>Runx2</i>	qPCR	Mm.PT.56a.31234632.g
<i>Sox9</i>	qPCR	Mm.PT.56a.42739087
<i>Sp7 (Osterix)</i>	qPCR	Mm.PT.56a.10898265
<i>Wnt4</i>	qPCR	Mm.PT.56a.8723468
<i>GAPDH (human)</i>	qPCR	Hs.PT.39a.22214836
<i>GREM1 (human)</i>	qPCR	Hs.PT.53a.26917089.g
<i>Acta2-RFP and R26-mT/mG</i>	Phenotyping	Carriers were detected by red fluorescence of the tail

Table 3A. Mice used in our study

Mouse line used	Reference	Received from	Description
<i>Grem1-creER^T</i>	Published here	Generated in the Wang lab.	A BAC transgenic in which the regulatory elements of <i>Grem1</i> drive expression of a tamoxifen inducible Cre recombinase. <i>Grem1</i> expressing cells express Cre recombinase that can, following binding of tamoxifen, translocate to the nucleus to recombine and thus activate fluorescent reporters or the diphtheria toxin subunit A lines described below.
<i>Grem1-LacZ</i>	Khokha et al., 2003	investigator	A LacZ reporter is knocked in to the endogenous <i>Grem1</i> locus. Cells that express <i>Grem1</i> can be detected by LacZ activity.
<i>Nes-creER^T</i>	Dranovsky et al., 2011	investigator	A transgenic line in which a short regulatory sequence of <i>Nes</i> drives expression of a tamoxifen inducible Cre recombinase. Following administration of tamoxifen, <i>Nes</i> expressing cells express Cre recombinase that can translocate to the nucleus to recombine and thus activate fluorescent reporters. In this line a 5.3kb region drives the CreERT2 upstream of the transcriptional start site of the Nestin gene. This fragment is fused to the CreERT2 followed by a polyA tail and then a 653bp region of the 2nd intron (corresponding to base pair positions 2880—>3533 downstream of the start site).
<i>Nes-GFP</i>	Mignone et al., 2004	investigator	A transgenic line in which a short regulatory sequence of <i>Nes</i> drives GFP expression.
<i>2.3ColGFP</i>	Kalajzic et al., 2002	JAX stock no. 13134	A transgenic line in which a short (2.3kb) regulatory sequence of rat <i>Col1a1</i> drives GFP expression. This line is used to identify committed osteoblasts in mice.
<i>Acta2-RFP</i>	Magness et al., 2004	investigator	A transgenic line in which a short regulatory sequence of <i>Acta2</i> drives RFP expression.
<i>R26-LSL-TdTomato</i>	Madisen et al., 2010	JAX stock no. 7909	A <i>cre</i> recombinase activated red fluorescent reporter knocked into the Rosa26 locus.
<i>R26-LSL-ZsGreen</i>	Madisen et al., 2010	JAX stock no. 7906	A <i>cre</i> recombinase activated green fluorescent reporter knocked into the Rosa26 locus.
<i>R26-LSL-mT/mG</i>	Muzumdar et al., 2007	JAX stock no. 7676	A <i>cre</i> recombinase activated green fluorescent reporter knocked into the Rosa26 locus. Prior to recombination all cells express a red fluorescent protein, which allows easier appreciation of tissue architecture on fluorescent microscopy.
<i>R26-LSL-Confetti</i>	Snippert et al., 2010	JAX stock no. 17492	A <i>cre</i> recombinase activated fluorescent reporter knocked into the Rosa26 locus. The value of this reporter is that one of 4 fluorescent tags is expressed randomly following recombination, either, YFP, RFP, mCFP or nGFP. This allows clones to be mapped <i>in vivo</i> .
<i>R26-LSL-DTA</i>	Voehringer et al., 2008	JAX stock no. 9669	A <i>cre</i> recombinase activated diphtheria toxin subunit A expressing construct knocked into the Rosa26 locus. This allowed cell specific programmed cell death.

Table 3B. Other mice previously reported as marking mesenchymal stem/progenitor cells, not included in our study

Mouse line used	Reported in	Description
<i>Lepr-cre</i>	Ding et al, 2012; Zhou et al 2014, Mizoguchi et al, 2014.	<i>Lepr-cre</i> is a knock-in, constitutive <i>Cre</i> line that primarily identifies perisinusoidal mesenchymal cells in the bone marrow. Across 3 papers the perisinusoidal cells it marks have been shown to not generate bone or cartilage in early post-natal life, but that in later life beginning around 2 months, <i>Lepr+</i> perisinusoidal cells begin to differentiate into bone and adipocytes and, in fracture callus, chondrocytes.
<i>Nes-Cre</i>	Mendez-Ferrer, et al, 2010- first published in Tronche et al, 1999	Lineage tracing of bone and cartilage has been reported from this line, albeit that the recombination may not perfectly mimic the perisinusoidal distribution of <i>Nes-GFP</i> .
<i>Nes-CreERT</i>	Mendez-Ferrer, et al, 2010-first published in Balordi & Fishell, 2007.	Lineage tracing of bone and cartilage has been reported from this line, albeit that the recombination may again not perfectly mimic the perisinusoidal distribution of <i>Nes-GFP</i> .
<i>Osx-Cre</i>	Mizoguchi et al, 2014. Maes et al, 2010, Park, et al 2012.	<i>Osterix (Sp7)</i> is a broad mesenchymal marker, but in adulthood is recognized as an early, but non-self-renewing, osteolineage marker. However, <i>Osx</i> is expressed in cell populations of varying potential throughout development and it was recently shown that perinatal <i>Osx</i> expressing cells (P5) are long-lived with bone and stromal potential.
<i>Ng2-CreERT</i>	Kunisaki et al, 2013; Feng, et al, 2011.	In the adult murine bone marrow <i>Ng2-CreERT^f</i> identifies periarterial cells, important for the HSC niche. In the tooth <i>Ng2+</i> cells are mesenchymal stem/progenitor cells generating odontoblasts.
<i>Acta2-CreER</i>	Grcevic et al, 2012.	<i>Acta2</i> expressing cells act as <i>in vitro</i> and <i>in vivo</i> mesenchymal stem/progenitor cells. <i>Acta2</i> is also expressed in smooth muscle and thus is probably expressed in both differentiated and less differentiated mesenchymal populations.
<i>Prx1-Cre</i>	Logan et al, 2002.	<i>Prx1</i> is expressed in early limb bud mesoderm and as a constitutive <i>Cre</i> will thus trace all lineages derived from limb bud mesoderm including bone, stroma and cartilage. It has been used in studies requiring broad mesodermal tracing and conditional knockouts.

Table 4 Bmp2 pathway Genes, Grem1+VsGrem1- fdr<.05

Symbol	Description	Log ₂ FC
Acvr1	activin A receptor, type 1	6.94
Bmp2	bone morphogenetic protein 2	5.79
Bmp5	bone morphogenetic protein 5	4.36
Bmp6	bone morphogenetic protein 6	2.18
Id2	inhibitor of DNA binding 2	2.56

Table 5		Grem1+ vs Grem1 - Significant KEGG Pathways		
Rank	Name	ID	pSize	onArray
1	ECM-receptor interaction	4512	87	85
2	PI3K-Akt signaling pathway	4151	356	333
3	Focal adhesion	4510	205	203
4	Fc gamma R-mediated phagocytosis	4666	89	87
5	Natural killer cell mediated cytotoxicity	4650	125	112
6	Osteoclast differentiation	4380	127	118
7	Amoebiasis	5146	120	113
8	Proteoglycans in cancer	5205	230	222
9	Cytokine-cytokine receptor interaction	4060	266	227
10	B cell receptor signaling pathway	4662	78	77
11	Regulation of actin cytoskeleton	4810	217	210
12	Aldosterone-regulated sodium reabsorption	4960	40	40
13	Fc epsilon RI signaling pathway	4664	71	70
14	MAPK signaling pathway	4010	259	253
15	HIF-1 signaling pathway	4066	113	104
16	VEGF signaling pathway	4370	66	66
17	Leishmaniasis	5140	66	66
18	Jak-STAT signaling pathway	4630	156	136
19	Transcriptional misregulation in cancer	5202	181	164
20	Axon guidance	4360	133	133
21	Phosphatidylinositol signaling system	4070	81	79
22	Long-term depression	4730	61	59
23	Tuberculosis	5152	179	170
24	Bacterial invasion of epithelial cells	5100	77	76
25	Salivary secretion	4970	77	73
26	Pancreatic secretion	4972	103	99
27	Leukocyte transendothelial migration	4670	121	116
28	Rheumatoid arthritis	5323	84	81
29	Viral myocarditis	5416	94	74
30	Renal cell carcinoma	5211	69	68
31	Staphylococcus aureus infection	5150	52	49
32	Glioma	5214	66	64
33	Hepatitis B	5161	149	140
34	Small cell lung cancer	5222	86	85
35	HTLV-I infection	5166	290	266
36	Salmonella infection	5132	79	77
37	Amphetamine addiction	5031	70	68
38	Viral carcinogenesis	5203	236	191
39	GnRH signaling pathway	4912	89	87

NDE	tA	pNDE	pPERT	pG	pG FDR	Status
26	4.91	4.E-12	0.00	1.E-08	2.E-06	Activated
48	2.53	3.E-08	0.02	3.E-07	2.E-05	Activated
49	2.36	6.E-17	0.02	4.E-07	2.E-05	Activated
27	1.53	1.E-12	0.12	2.E-06	7.E-05	Not Applicable
21	2.12	4.E-06	0.04	2.E-06	7.E-05	Activated
23	0.94	7.E-07	0.34	5.E-06	1.E-04	Not Applicable
25	0.65	2.E-08	0.52	8.E-06	2.E-04	Not Applicable
36	0.44	9.E-08	0.66	1.E-05	2.E-04	Not Applicable
32	-1.70	1.E-05	0.08	1.E-05	2.E-04	Not Applicable
18	0.13	7.E-07	0.89	1.E-05	2.E-04	Not Applicable
34	-0.10	2.E-07	0.92	1.E-05	2.E-04	Not Applicable
11	-0.46	2.E-05	0.67	2.E-04	2.E-03	Not Applicable
14	1.00	8.E-05	0.30	3.E-04	3.E-03	Not Applicable
33	0.07	4.E-05	0.94	4.E-04	4.E-03	Not Applicable
16	1.71	6.E-04	0.07	5.E-04	4.E-03	Not Applicable
13	-1.06	2.E-04	0.28	5.E-04	4.E-03	Not Applicable
13	0.88	2.E-04	0.36	6.E-04	5.E-03	Not Applicable
20	-0.76	2.E-04	0.46	1.E-03	9.E-03	Not Applicable
22	-0.47	5.E-04	0.34	2.E-03	0.01	Not Applicable
19	-0.83	5.E-04	0.40	2.E-03	0.01	Not Applicable
13	-0.73	1.E-03	0.30	3.E-03	0.02	Not Applicable
9	2.11	9.E-03	0.04	3.E-03	0.02	Activated
22	0.67	8.E-04	0.49	3.E-03	0.02	Not Applicable
13	-0.36	7.E-04	0.72	4.E-03	0.02	Not Applicable
12	-0.68	2.E-03	0.50	6.E-03	0.03	Not Applicable
15	-0.24	1.E-03	0.82	7.E-03	0.04	Not Applicable
16	0.71	2.E-03	0.46	7.E-03	0.04	Not Applicable
11	1.53	1.E-02	0.09	7.E-03	0.04	Not Applicable
12	-0.61	2.E-03	0.54	8.E-03	0.04	Not Applicable
11	-0.88	3.E-03	0.39	8.E-03	0.04	Not Applicable
8	-1.43	9.E-03	0.12	9.E-03	0.04	Not Applicable
10	-1.11	5.E-03	0.22	9.E-03	0.04	Not Applicable
18	-0.62	2.E-03	0.53	1.E-02	0.04	Not Applicable
12	1.19	6.E-03	0.23	1.E-02	0.04	Not Applicable
29	0.41	2.E-03	0.68	1.03E-02	0.040327256	Not Applicable
12	0.44	2.E-03	0.65	1.20E-02	0.045777725	Not Applicable
11	0.49	3.E-03	0.63	1.26E-02	0.0467475	Not Applicable
22	-0.70	3.E-03	0.57	1.41E-02	0.049467655	Not Applicable
12	0.98	7.E-03	0.29	1.41E-02	0.049467655	Not Applicable

Table 6. Grem1+ vs Grem1 - Significant Reactome Pathways

Rank	Name
1	Collagen biosynthesis and modifying enzymes
2	MPS IIIC - Sanfilippo syndrome C
3	MPS IV - Morquio syndrome B
4	MPS VI - Maroteaux-Lamy syndrome
5	MPS II - Hunter syndrome
6	Glycosaminoglycan metabolism
7	MPS IX - Natowicz syndrome
8	MPS VII - Sly syndrome
9	MPS I - Hurler syndrome
10	MPS IIIB - Sanfilippo syndrome B
11	MPS IIIA - Sanfilippo syndrome A
12	MPS IIID - Sanfilippo syndrome D
13	MPS IV - Morquio syndrome A
14	Fcγ receptor (FCGR) dependent phagocytosis
15	Cell surface interactions at the vascular wall
16	Collagen degradation
17	Integrin cell surface interactions
18	Assembly of collagen fibrils and other multimeric structures
19	Degradation of the extracellular matrix
20	Signaling by Rho GTPases
21	GPVI-mediated activation cascade
22	Response to elevated platelet cytosolic Ca ²⁺
23	Syndecan interactions
24	Elastic fibre formation
25	Signaling by Hippo
26	Activation of Matrix Metalloproteinases
27	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell
28	Regulation of lipid metabolism by Peroxisome proliferator-activated receptor alpha (PPARα)

ID	pSize	onArray	NDE	tA	pNDE	pPERT	pG	pG FDR	Status
5605856	52	50	20	1.96	0.E+00	0.05	0.00	7.E-05	Activated
5605255	110	108	25	-0.98	0.E+00	0.32	0.00	7.E-05	Not Applicable
5605261	110	108	25	-0.96	0.E+00	0.33	0.00	7.E-05	Not Applicable
5605253	110	108	25	-0.96	0.E+00	0.34	0.00	7.E-05	Not Applicable
5605257	110	108	25	-0.95	0.E+00	0.34	0.00	7.E-05	Not Applicable
5605249	110	108	25	-0.96	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605250	110	108	25	-0.95	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605256	110	108	25	-0.94	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605258	110	108	25	-0.94	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605252	110	108	25	-0.94	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605260	110	108	25	-0.94	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605259	110	108	25	-0.93	0.E+00	0.35	0.00	7.E-05	Not Applicable
5605254	110	108	25	-0.95	0.E+00	0.37	0.00	7.E-05	Not Applicable
5605170	72	71	17	-0.53	1.E-06	0.61	0.00	1.E-04	Not Applicable
5605041	83	81	17	1.45	7.E-06	0.13	0.00	1.E-04	Not Applicable
5605823	31	31	10	1.47	1.E-05	0.13	0.00	2.E-04	Not Applicable
5605038	60	58	14	1.00	8.E-06	0.34	0.00	3.E-04	Not Applicable
5605828	27	27	9	-0.91	2.E-05	0.56	0.00	1.E-03	Not Applicable
5605824	94	87	16	-1.05	7.E-05	0.28	0.00	2.E-03	Not Applicable
5605351	100	97	15	1.77	8.E-04	0.06	0.00	4.E-03	Not Applicable
5605042	26	26	8	0.21	1.E-04	0.83	0.00	0.01	Not Applicable
5605037	86	81	14	-0.17	4.E-04	0.93	0.00	0.02	Not Applicable
5605962	18	18	6	0.37	5.E-04	0.74	0.00	0.02	Not Applicable
5605850	36	36	7	-1.32	6.E-03	0.12	0.01	0.03	Not Applicable
5605905	20	20	6	0.29	1.E-03	0.80	0.01	0.04	Not Applicable
5605848	45	39	8	0.93	2.E-03	0.36	0.01	0.04	Not Applicable
5605383	51	43	7	1.66	1.E-02	0.07	0.01	0.04	Not Applicable
5605592	73	73	11	-1.10	5.E-03	0.23	0.01	0.04	Not Applicable

Table 7 ECM-Receptor Interaction Genes in
Grem1+ Vs Grem1- (fdr<0.05)

Symbol	Description	Log ₂ FC
Chad	chondroadherin	5.39
Col11a1	collagen, type XI, alpha 1	3.77
Col11a2	collagen, type XI, alpha 2	4.14
Col27a1	collagen, type XXVII, alpha 1	2.88
Col4a2	collagen, type IV, alpha 2	1.49
Col4a5	collagen, type IV, alpha 5	4.27
Col5a1	collagen, type V, alpha 1	3.09
Col5a2	collagen, type V, alpha 2	2.49
Col6a1	collagen, type VI, alpha 1	5.01
Col6a2	collagen, type VI, alpha 2	4.00
Col6a3	collagen, type VI, alpha 3	4.66
Comp	cartilage oligomeric matrix protein	4.00
Dag1	dystroglycan 1	2.35
Hspg2	perlecan (heparan sulfate proteoglycan 2)	2.60
Ibsp	integrin binding sialoprotein	3.26
Itga4	integrin alpha 4	-4.24
Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
Itga6	integrin alpha 6	2.17
Itgav	integrin alpha V	2.81
Itgb3	integrin beta 3	-1.62
Lama4	laminin, alpha 4	1.80
Lamb2	laminin, beta 2	2.50
Npnt	nephronectin	3.91
Sdc1	syndecan 1	2.76
Sdc4	syndecan 4	4.91
Thbs3	thrombospondin 3	4.57

	A	B	C
1	Table 8	PI3K-Akt Signaling Genes in	
2		Grem1+ Vs Grem1- (fdr<0.05)	
3	Symbol	Description	Log ₂ FC
4	Ccnd1	cyclin D1	4.35
5	Ccnd3	cyclin D3	-2.04
6	Chad	chondroadherin	5.39
7	Col11a1	collagen, type XI, alpha 1	3.77
8	Col11a2	collagen, type XI, alpha 2	4.14
9	Col27a1	collagen, type XXVII, alpha 1	2.88
10	Col4a2	collagen, type IV, alpha 2	1.49
11	Col4a5	collagen, type IV, alpha 5	4.27
12	Col5a1	collagen, type V, alpha 1	3.09
13	Col5a2	collagen, type V, alpha 2	2.49
14	Col6a1	collagen, type VI, alpha 1	5.01
15	Col6a2	collagen, type VI, alpha 2	4.00
16	Col6a3	collagen, type VI, alpha 3	4.66
17	Comp	cartilage oligomeric matrix protein	4.00
18	Creb3l2	cAMP responsive element binding protein 3-like 2	4.85
19	Csf1r	colony stimulating factor 1 receptor	-1.73
20	Fgf2	fibroblast growth factor 2	5.70
21	Fgfr1	fibroblast growth factor receptor 1	2.60
22	Fgfr2	fibroblast growth factor receptor 2	5.65
23	Fgfr3	fibroblast growth factor receptor 3	3.86
24	Ibsp	integrin binding sialoprotein	3.26
25	Igf1	insulin-like growth factor 1	4.11
26	Itga4	integrin alpha 4	-4.24
27	Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
28	Itga6	integrin alpha 6	2.17
29	Itgav	integrin alpha V	2.81
30	Itgb3	integrin beta 3	-1.62
31	Lama4	laminin, alpha 4	1.80
32	Lamb2	laminin, beta 2	2.50
33	Lpar1	lysophosphatidic acid receptor 1	3.03
34	Lpar4	lysophosphatidic acid receptor 4	4.46
35	Lpar6	lysophosphatidic acid receptor 6	-3.83
36	Mapk1	mitogen-activated protein kinase 1	-3.32
37	Myb	myeloblastosis oncogene	-5.06
38	Ngf	nerve growth factor	5.93
39	Osmr	oncostatin M receptor	5.40
40	Pdgfa	platelet derived growth factor, alpha	1.50
41	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	-2.58
42	Pten	phosphatase and tensin homolog	-3.97
43	Ptk2	PTK2 protein tyrosine kinase 2	2.83
44	Rheb	Ras homolog enriched in brain	1.33
45	Sgk1	serum/glucocorticoid regulated kinase 1	2.45
46	Sos2	son of sevenless homolog 2 (Drosophila)	-2.70
47	Syk	spleen tyrosine kinase	-4.15
48	Thbs3	thrombospondin 3	4.57
49	Tlr4	toll-like receptor 4	-2.88
50	Vegfa	vascular endothelial growth factor A	1.87
51	Ywhaq	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	1.77

Table 9	Focal Adhesion Genes in Grem1+ Vs Grem1- (fdr<0.05)	
Symbol	Description	Log ₂ FC
Actn4	actinin alpha 4	1.47
Capn2	calpain 2	3.45
Cav1	caveolin 1, caveolae protein	3.07
Cav2	caveolin 2	3.08
Ccnd1	cyclin D1	4.35
Ccnd3	cyclin D3	-2.04
Chad	chondroadherin	5.39
Col11a1	collagen, type XI, alpha 1	3.77
Col11a2	collagen, type XI, alpha 2	4.14
Col27a1	collagen, type XXVII, alpha 1	2.88
Col4a2	collagen, type IV, alpha 2	1.49
Col4a5	collagen, type IV, alpha 5	4.27
Col5a1	collagen, type V, alpha 1	3.09
Col5a2	collagen, type V, alpha 2	2.49
Col6a1	collagen, type VI, alpha 1	5.01
Col6a2	collagen, type VI, alpha 2	4.00
Col6a3	collagen, type VI, alpha 3	4.66
Comp	cartilage oligomeric matrix protein	4.00
Flnb	filamin, beta	2.89
Flnc	filamin C, gamma	3.35
Ibsp	integrin binding sialoprotein	3.26
Igf1	insulin-like growth factor 1	4.11
Itga4	integrin alpha 4	-4.24
Itga5	integrin alpha 5 (fibronectin receptor alpha)	3.73
Itga6	integrin alpha 6	2.17
Itgav	integrin alpha V	2.81
Itgb3	integrin beta 3	-1.62
Jun	jun proto-oncogene	1.98
Lama4	laminin, alpha 4	1.80
Lamb2	laminin, beta 2	2.50
Mapk1	mitogen-activated protein kinase 1	-3.32
Mylk	myosin, light polypeptide kinase	2.14
Pak3	p21 protein (Cdc42/Rac)-activated kinase 3	5.49
Parvg	parvin, gamma	-3.07
Pdgfa	platelet derived growth factor, alpha	1.50
Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	-2.58
Prkcb	protein kinase C, beta	-3.85
Prkcg	protein kinase C, gamma	2.75

Pten	phosphatase and tensin homolog	-3.97
Ptk2	PTK2 protein tyrosine kinase 2	2.83
Rac2	RAS-related C3 botulinum substrate 2	-3.03
Sos2	son of sevenless homolog 2 (Drosophila)	-2.70
Thbs3	thrombospondin 3	4.57
Vasp	vasodilator-stimulated phosphoprotein	-1.39
Vav1	vav 1 oncogene	-4.46
Vav2	vav 2 oncogene	2.38
Vav3	vav 3 oncogene	-2.01
Vegfa	vascular endothelial growth factor A	1.87
Xiap	X-linked inhibitor of apoptosis	-2.45

Table 10	Osteoblast differentiation GO:0001649			
Symbol	probeid	logFC	P.Value	fdr
Shox2	1438042_at	7.13969309	1.12E-05	0.02471733
Fgfr2	1433489_s_a	5.65231788	1.51E-05	0.02471733
Bmp2	1423635_at	5.78726506	5.84E-05	0.02471733
Col9a1	1421381_a_a	5.14384919	7.20E-05	0.02471733
Sox9	1424950_at	6.90133905	0.0001102	0.02471733
Comp	1419527_at	3.99952788	0.00013709	0.02523526
Papss2	1421987_at	5.85768973	0.00013725	0.02523526
Col10a1	1422253_at	4.64970077	0.00015096	0.02523526
Matn1	1418477_at	5.63933284	0.00019186	0.02642403
Impad1	1437290_at	4.00009567	0.00019808	0.02656323
Ltbp3	1437833_at	2.9661723	0.00021743	0.02704011
Sp7	1418425_at	3.10351174	0.00026166	0.02862484
Ddr2	1422738_at	3.89722768	0.00026991	0.02873843
Ankrd11	1458452_at	-1.7496439	0.00042781	0.03083902
Bmp6	1450759_at	2.1776606	0.00052763	0.03237488
Thbs3	1416623_at	4.56764372	0.00056966	0.03323698
Ptprc	1440165_at	-5.6734651	0.00064536	0.03437141
Igf1	1419519_at	4.10582922	0.00076686	0.03552034
Mef2c	1451506_at	1.5622972	0.00077261	0.03552034
Gli3	1456067_at	2.45472986	0.00081314	0.0360597
Insig1	1454671_at	2.08360386	0.00085701	0.03646133
Csgalnact1	1452365_at	6.10211317	0.00087003	0.03646133
Asxl2	1460597_at	-2.6979367	0.00092542	0.0368767
Hspg2	1418670_s_a	2.60149201	0.00098897	0.03770361
Dlx5	1449863_a_a	3.7588245	0.00106207	0.03918556
Sparc	1416589_at	1.60412839	0.00113447	0.04021149
Slc38a10	1427295_at	1.83634246	0.0012518	0.04142547
Has2	1449169_at	4.99235834	0.00126234	0.04164276
Fgfr3	1421841_at	3.86261057	0.00148679	0.04425268
Npr2	1427191_at	3.46860457	0.00168539	0.04660501
Sulf2	1442408_at	1.6036124	0.00181894	0.04807212
Serpinh1	1450843_a_a	1.29654547	0.00188638	0.04851906
Sema4d	1420824_at	-1.8029043	0.00194795	0.04888768
Phospho2	1425190_a_a	-2.8140356	0.00197866	0.04910038
Alpl	1423611_at	2.96345394	0.00315219	0.05979792
Cadm1	1417376_a_a	4.10590728	0.00374974	0.06413052
Smad1	1448208_at	3.17101958	0.00393954	0.06521738
Fat4	1459749_s_a	3.23899393	0.00402532	0.06597077
Runx2	1424704_at	2.8878129	0.0055077	0.0751405

Rarg	1419415_a_a	1.55028671	0.0058013	0.07697684
Insig2	1417980_a_a	2.44324681	0.00584458	0.07727179
Sik3	1460439_at	-1.0857648	0.00755719	0.08777671
Cyp26b1	1460011_at	2.41494558	0.0078034	0.0892175
Glg1	1460554_s_a	1.08231524	0.00954803	0.09811991
Pex7	1418988_at	-1.5114512	0.01007962	0.1014008
Gnas	1450186_s_a	1.15793611	0.01017669	0.1019462
Smad5	1433641_at	1.32605317	0.01176342	0.10947652
Ostc	1449139_at	1.47773534	0.01312531	0.1164395
Plxnb1	1435254_at	2.94240991	0.01342921	0.11774318
Bbx	1425835_a_a	2.44695116	0.01448366	0.12264883
Twist1	1418733_at	1.38261178	0.01576956	0.1278689
Nab1	1438819_at	-2.9216854	0.01616797	0.1294138
Asxl1	1458380_at	-1.3363295	0.02003387	0.1445676
Osr2	1426155_a_a	0.50221439	0.02482068	0.16093223
Setdb1	1451833_a_a	-0.705544	0.02602998	0.16493091
Pthlh	1422324_a_a	2.22023888	0.02765368	0.17061675
Ift80	1427568_a_a	2.13652764	0.03549998	0.19425924
Col2a1	1450567_a_a	0.51632868	0.03698764	0.19875846
Cited2	1452207_at	0.77720105	0.038182	0.20226056
Pax1	1449359_at	3.12937179	0.03911755	0.20500123
Ski	1426373_at	1.20823577	0.03974484	0.20676946
Rhoa	1437628_s_a	-0.4614252	0.04469134	0.22031089
Bnc2	1438861_at	4.23430725	0.04850308	0.23058618
Whsc1	1435136_at	-1.0033239	0.06021278	0.25860931
Inpp1	1460394_a_a	0.56759927	0.06832218	0.27657199
Mcph1	1439115_at	-1.2317502	0.07426703	0.289485
Fgf18	1449545_at	1.95630563	0.08013069	0.30080298
Hoxa11	1420414_at	0.51117163	0.134279	0.39421853
Ctc1	1423656_x_a	-0.3454466	0.13779297	0.40027058
Sh3pxd2b	1442919_at	0.7836116	0.1378523	0.40031788
Evc	1448876_at	1.72036865	0.14073692	0.40431581
Lrp6	1451022_at	1.84723464	0.14520466	0.41054822
Grem1	1425357_a_a	2.03742601	0.15410452	0.42297152
Amer1	1439565_at	-1.2063171	0.16004689	0.43090105
Scx	1456291_x_a	0.27534435	0.16950076	0.44294863
Ptger4	1424208_at	-0.9729169	0.17847174	0.45532605
Ryr1	1427306_at	-0.2950732	0.19148114	0.47135849
Rab23	1454876_at	0.859713	0.19401749	0.47439891
Trim45	1441412_s_a	0.20342791	0.20276127	0.48402773
Axin2	1436845_at	0.38799213	0.21124748	0.49331913

Lrrc17	1429679_at	0.23301036	0.23562804	0.52060256
Pitx2	1424797_a_a	1.07081967	0.23628235	0.52138414
Carm1	1419743_s_a	0.64722551	0.2531193	0.53909773
Dym	1423736_a_a	-0.9150064	0.27126619	0.55550201
Sulf1	1436319_at	1.19568804	0.27432954	0.5581008
Smad9	1450265_at	0.16221583	0.2789619	0.56241214
Prpsap2	1452062_at	-0.3149736	0.28405301	0.56696207
Msx2	1438351_at	0.35290248	0.29922583	0.58127152
Mef2d	1421388_at	-0.1866246	0.31750884	0.59885034
Nppc	1422790_at	0.1607409	0.31851616	0.59964979
Fam73b	1454621_s_a	0.2994231	0.32117143	0.60193506
Acp5	1431609_a_a	-0.9309661	0.33111888	0.61004404
Fgf4	1420086_x_a	0.18263932	0.35648536	0.63248628
Cbs	1423844_s_a	0.20063418	0.36616507	0.64041613
Wnt1	1425377_at	0.36975782	0.37967184	0.65054435
T	1419304_at	0.16388178	0.39058468	0.65912444
Col1a1	1423669_at	-0.6212641	0.40659023	0.6718836
Hoxb4	1451761_at	-0.1272661	0.46260484	0.71610719
Sp5	1422914_at	-0.1174096	0.4953465	0.73863064
Hoxd11	1450584_at	0.09335222	0.51678271	0.75267603
Bmp4	1422912_at	-0.1515746	0.52844446	0.76072052
Fgf8	1451882_a_a	0.08277176	0.56350817	0.78452791
Rarb	1454906_at	0.13246962	0.58074285	0.79545915
Msx1	1417127_at	-0.0792768	0.6088118	0.81219928
Por	1416933_at	-0.0809813	0.64020848	0.83040587
Thbs1	1460302_at	-0.0940899	0.6403299	0.83041932
Rara	1450180_a_a	-0.1450325	0.65688912	0.83924187
Lrp5	1449299_at	0.12006994	0.66717382	0.84534924
Tfap2a	1421996_at	0.04882766	0.73011248	0.87883831
Lep	1422582_at	-0.051409	0.74333518	0.88629937
Spns2	1451601_a_a	-0.0930905	0.75077046	0.89005592
Nab2	1417930_at	-0.1414283	0.76360144	0.89720433
Sfrp2	1448201_at	0.26766348	0.76534268	0.89810537
Dchs1	1429163_at	0.03405801	0.85190273	0.93849266
Sbds	1426480_at	-0.0651239	0.88207204	0.95094397
Bglap2	1449880_s_a	0.03089977	0.96146152	0.98359981
Cdx1	1449582_at	-0.0012411	0.99265715	0.99719485
Frem1	1455280_at	0.0010303	0.99394523	0.99782363

Table 11 Chondrocyte differentiation GO:0002062

Symbol	probeid	logFC	P.Value	globalfdr
Sox5	1452511_at	7.22767132	7.96E-06	0.02471733
Frzb	1416658_at	6.48749238	8.83E-06	0.02471733
Shox2	1438042_at	7.13969309	1.12E-05	0.02471733
Trps1	1438214_at	3.40755204	4.76E-05	0.02471733
Cytl1	1456793_at	6.87459727	5.09E-05	0.02471733
Bmp2	1423635_at	5.78726506	5.84E-05	0.02471733
Col11a2	1423578_at	4.14206689	6.46E-05	0.02471733
Pkdcc	1454838_s_a	7.28962976	6.74E-05	0.02471733
Col9a1	1421381_a_e	5.14384919	7.20E-05	0.02471733
Sox9	1424950_at	6.90133905	0.0001102	0.02471733
Tgfb2	1423250_a_e	5.54649939	0.00012244	0.02520744
Comp	1419527_at	3.99952788	0.00013709	0.02523526
Col10a1	1422253_at	4.64970077	0.00015096	0.02523526
Matn1	1418477_at	5.63933284	0.00019186	0.02642403
Impad1	1437290_at	4.00009567	0.00019808	0.02656323
Sox6	1447655_x_a	3.04224037	0.00020449	0.0267325
Ltbp3	1437833_at	2.9661723	0.00021743	0.02704011
Nfib	1434101_at	2.200371	0.00030068	0.02873843
Chst11	1450509_at	4.02884726	0.00032235	0.02907679
Creb3l2	1452381_at	4.85293127	0.00046023	0.03115804
Bmp6	1450759_at	2.1776606	0.00052763	0.03237488
Col11a1	1418599_at	2.34126506	0.00053144	0.03247748
Thbs3	1416623_at	4.56764372	0.00056966	0.03323698
Mmp13	1417256_at	4.24452375	0.00061702	0.03397116
Cyr61	1438133_a_e	5.69119846	0.00063681	0.03419138
Mia3	1459984_at	2.37058997	0.00065816	0.03447572
Hif1a	1448183_a_e	1.72827087	0.00074424	0.03532241
Bmp5	1455851_at	4.3611194	0.00075454	0.03535411
Mef2c	1451506_at	1.5622972	0.00077261	0.03552034
Fgf2	1449826_a_e	5.70455759	0.00078373	0.03566748
Gli3	1456067_at	2.45472986	0.00081314	0.0360597
Pth1r	1417092_at	5.14446867	0.00081315	0.0360597
Acan	1449827_at	3.41833726	0.0008224	0.03611613
Fgfr1	1424050_s_a	2.60129371	0.00086507	0.03646133
Csgalnact1	1452365_at	6.10211317	0.00087003	0.03646133
Hspg2	1418670_s_a	2.60149201	0.00098897	0.03770361
Mgp	1448416_at	3.3507084	0.00116189	0.0404339
Snai2	1418673_at	5.03701152	0.00128582	0.04171829
Fgfr3	1421841_at	3.86261057	0.00148679	0.04425268

Maf	1437473_at	3.76980305	0.00153687	0.04489271
Ctgf	1416953_at	2.49942916	0.00172491	0.04701858
Sulf2	1442408_at	1.6036124	0.00181894	0.04807212
Serpinh1	1450843_a_ε	1.29654547	0.00188638	0.04851906
Thra	1443952_at	1.82594965	0.00237175	0.05300708
Zbtb16	1419874_x_a	3.80377306	0.00239811	0.05325226
Bmp8a	1449873_at	2.20192492	0.00384863	0.06493723
Smad1	1448208_at	3.17101958	0.00393954	0.06521738
Pkd1	1460210_at	2.9085668	0.00405261	0.06613797
Bmp7	1418910_at	1.92696736	0.00418937	0.06714193
Mex3c	1444701_at	2.84275529	0.00439563	0.06871649
Bmpr1a	1425492_at	1.36200773	0.00516927	0.07349907
Runx2	1424704_at	2.8878129	0.0055077	0.0751405
Rarg	1419415_a_ε	1.55028671	0.0058013	0.07697684
Barx2	1421761_a_ε	0.85624097	0.00640975	0.08068276
Tgfb1	1445360_at	-1.8533881	0.00659249	0.08152761
Lect1	1460258_at	4.58986317	0.00697288	0.08388477
Ctnnb1	1450008_a_ε	1.66261927	0.00720075	0.08544095
Tgfbr2	1426397_at	1.86659547	0.0075086	0.08755963
Sik3	1460439_at	-1.0857648	0.00755719	0.08777671
Hoxc4	1422870_at	1.19796075	0.00761056	0.08803383
Otor	1425083_at	4.22357952	0.00876964	0.09435106
Ror2	1457128_at	4.33909419	0.008963	0.09531397
Glg1	1460554_s_a	1.08231524	0.00954803	0.09811991
Tgfbr1	1420893_a_ε	-1.553593	0.00976607	0.09956142
Gnas	1450186_s_a	1.15793611	0.01017669	0.1019462
Smad5	1433641_at	1.32605317	0.01176342	0.10947652
Prkca	1427562_a_ε	2.10613284	0.01358718	0.11834592
Hes5	1456010_x_a	-2.1231445	0.01655693	0.13093705
Gli2	1459211_at	1.04059295	0.0176753	0.13518291
Mapk14	1426104_at	-1.5532261	0.02097546	0.14796945
Lnp	1453035_at	-2.9755281	0.02392091	0.15807429
Osr2	1426155_a_ε	0.50221439	0.02482068	0.16093223
Hmga2	1450781_at	1.09586861	0.02694941	0.16831614
Pthlh	1422324_a_ε	2.22023888	0.02765368	0.17061675
Wnt9a	1436978_at	0.87174088	0.02785817	0.17122255
Bmpr1b	1437312_at	1.25973479	0.02801041	0.17173704
Prrx1	1432129_a_ε	1.47773595	0.0280624	0.17191557
Foxd1	1418876_at	4.46730525	0.03043986	0.17907112
Ift80	1427568_a_ε	2.13652764	0.03549998	0.19425924
Col2a1	1450567_a_ε	0.51632868	0.03698764	0.19875846

Thrb	1422202_at	2.48066506	0.04363391	0.21759789
Atp7a	1418774_a_ε	-1.6739022	0.04473941	0.22045143
Mapk3	1427060_at	-0.665697	0.04913025	0.23233967
Wnt5a	1448818_at	2.93263031	0.0492736	0.23264251
Bmp1	1427457_a_ε	2.43028686	0.05127348	0.23736193
Rela	1419536_a_ε	1.04767874	0.05767973	0.25345212
Hoxa5	1443803_x_a	1.0275933	0.06149235	0.26170129
Esrra	1442864_at	-0.6446893	0.06725047	0.27435072
Fgf18	1449545_at	1.95630563	0.08013069	0.30080298
Hoxa3	1427433_s_a	0.507085	0.08120338	0.30287153
Zbtb7a	1437255_at	-0.3664643	0.09699201	0.33330316
Runx3	1440275_at	0.96780328	0.09793019	0.33494632
Hoxb3	1427605_at	0.39418388	0.09973918	0.33804288
Edn1	1451924_a_ε	-0.2982996	0.10195385	0.34187512
Eif2ak3	1430371_x_a	-1.4225496	0.11979267	0.37163913
Satb2	1425904_at	0.48646604	0.12821542	0.38470549
Bbs1	1437310_at	1.90386954	0.12829484	0.38470549
Hoxa11	1420414_at	0.51117163	0.134279	0.39421853
Bmp8b	1440706_at	0.26308768	0.13555736	0.39655082
Foxd2	1442315_at	0.46320213	0.14384418	0.40876544
Lrp6	1451022_at	1.84723464	0.14520466	0.41054822
Zeb1	1418926_at	1.16354749	0.14660325	0.41268652
Fgf9	1438718_at	-0.3966601	0.15928736	0.42979473
Scx	1456291_x_a	0.27534435	0.16950076	0.44294863
Wnt7a	1458334_at	0.23058566	0.17829566	0.45510852
Mkks	1422627_a_ε	0.62949897	0.20423492	0.48574462
Bbs2	1424478_at	1.18847208	0.20451523	0.48587449
Axin2	1436845_at	0.38799213	0.21124748	0.49331913
Arid5a	1451340_at	-0.3128111	0.21194496	0.49438477
Hand1	1417525_at	0.20127201	0.24088701	0.52659659
Carm1	1419743_s_a	0.64722551	0.2531193	0.53909773
Sulf1	1436319_at	1.19568804	0.27432954	0.5581008
Smad9	1450265_at	0.16221583	0.2789619	0.56241214
Mycn	1417155_at	0.39769166	0.2893957	0.57218164
Pitx1	1419514_at	0.1702969	0.29146369	0.57395417
Msx2	1438351_at	0.35290248	0.29922583	0.58127152
Prrx2	1432331_a_ε	0.73191217	0.30091849	0.58270253
Wnt7b	1420892_at	0.15715266	0.31454776	0.59632366
Mef2d	1421388_at	-0.1866246	0.31750884	0.59885034
Nppc	1422790_at	0.1607409	0.31851616	0.59964979
Chrdl2	1420539_a_ε	0.20332488	0.34147543	0.61922033

Uncx	1419633_at	0.14875918	0.35394085	0.62995605
Fgf4	1420086_x_a	0.18263932	0.35648536	0.63248628
Cbs	1423844_s_a	0.20063418	0.36616507	0.64041613
Cst10	1449447_at	0.19712187	0.36842711	0.6420478
Gdf5	1419139_at	0.12908806	0.37130522	0.6441108
Foxd4	1422318_at	-0.1344761	0.3797373	0.65058051
Foxd3	1422210_at	0.12298744	0.39927456	0.66574299
Col1a1	1423669_at	-0.6212641	0.40659023	0.6718836
Fgf6	1427582_at	0.13306027	0.43564081	0.69508034
Nog	1422300_at	0.1532112	0.44376711	0.70129789
Smad3	1450472_s_a	0.28466688	0.44828933	0.70498612
Fbxw4	1417226_at	0.15445078	0.48947258	0.73441242
Six2	1427436_at	0.11798718	0.49326442	0.73685768
Dlx2	1448877_at	0.17993045	0.50144031	0.74322059
Hoxd11	1450584_at	0.09335222	0.51678271	0.75267603
Bmp4	1422912_at	-0.1515746	0.52844446	0.76072052
Rarb	1454906_at	0.13246962	0.58074285	0.79545915
Msx1	1417127_at	-0.0792768	0.6088118	0.81219928
Pax7	1452510_at	-0.0726034	0.61587116	0.81680712
Por	1416933_at	-0.0809813	0.64020848	0.83040587
Thbs1	1460302_at	-0.0940899	0.6403299	0.83041932
Rara	1450180_a_e	-0.1450325	0.65688912	0.83924187
Osr1	1449350_at	0.06674263	0.65796737	0.83979472
Nkx3-2	1421464_at	0.07259392	0.65988471	0.84131336
Snai1	1448742_at	0.051586	0.71227826	0.86945623
Hand2	1436041_at	0.05183208	0.71914945	0.87278294
Lep	1422582_at	-0.051409	0.74333518	0.88629937
Sfrp2	1448201_at	0.26766348	0.76534268	0.89810537
Myf5	1420757_at	0.03687913	0.79843488	0.91361693
Ihh	1450704_at	0.03067369	0.82163053	0.9243161
Efemp1	1427183_at	0.22630155	0.84645157	0.93572931
Hoxd3	1421537_at	-0.0194301	0.90332787	0.96012428
Rspo2	1455893_at	-0.0229601	0.96084842	0.9833494

Table 12 Adipocyte Differentiation

GO:0045444

Symbol	probeid	logFC	P.Value	fdr
Frzb	1416658_at	6.48749238	8.83E-06	0.02471733
Scd1	1415965_at	6.92946129	2.02E-05	0.02471733
Wif1	1425425_a_a	5.61016474	2.97E-05	0.02471733
Wwtr1	1417818_at	4.26966834	4.04E-05	0.02471733
Bmp2	1423635_at	5.78726506	5.84E-05	0.02471733
Medag	1452244_at	2.54509537	0.00012336	0.02520744
Fndc3b	1433833_at	4.14105154	0.00016123	0.02523526
Id2	1435176_a_a	2.55980464	0.00017265	0.02523526
Enpp1	1419276_at	4.18103881	0.0001804	0.025505
Ccnd1	1448698_at	4.35237065	0.0006197	0.03397116
Selenbp1	1450699_at	-2.0726353	0.00069184	0.03471676
Plcb1	1435043_at	4.48210625	0.00069203	0.03471676
Igf1	1419519_at	4.10582922	0.00076686	0.03552034
4932438A13f	1444660_at	-1.7217063	0.00083553	0.03627182
Insig1	1454671_at	2.08360386	0.00085701	0.03646133
Asxl2	1460597_at	-2.6979367	0.00092542	0.0368767
Itga6	1422445_at	2.16501935	0.00095162	0.03712712
Ero1l	1449324_at	-5.2415423	0.00109722	0.03974742
Klf4	1417394_at	2.49143131	0.00117445	0.04059755
Snai2	1418673_at	5.03701152	0.00128582	0.04171829
Lama4	1424807_at	1.79871601	0.00144798	0.04381912
Plac8	1451335_at	-3.9343763	0.00197629	0.04910038
Zbtb16	1419874_x_a	3.80377306	0.00239811	0.05325226
Rgs2	1419248_at	-2.0621651	0.00309171	0.05930885
Id4	1423259_at	3.91112981	0.00323253	0.06026486
Egr2	1427683_at	2.26204995	0.00334336	0.06120178
Gpx1	1460671_at	-1.5018199	0.00419115	0.06714193
1100001G20l	1434484_at	-2.8361979	0.00426222	0.06782998
Zfpm2	1449314_at	2.05338287	0.0043193	0.06807548
Mex3c	1444701_at	2.84275529	0.00439563	0.06871649
Lamb3	1417812_a_a	1.6568793	0.00481526	0.07143995
Osbp18	1437069_at	-1.0291454	0.00523485	0.07380639
Tcf7l2	1429428_at	2.81979243	0.00526505	0.07387511
Fcor	1439834_at	-1.0060617	0.00590615	0.07772749
Nipbl	1442103_at	-1.2510927	0.00646363	0.08095419
Tgfb1	1445360_at	-1.8533881	0.00659249	0.08152761
Psmb8	1422962_a_a	-2.8058177	0.00674644	0.08249817
Creb1	1428755_at	-2.7091354	0.00842715	0.09235384
Bnip3	1422470_at	4.08594527	0.00951743	0.09797892

Creb5	1457222_at	2.78212192	0.01161505	0.10890856
Akt1	1425711_a_a	0.64331207	0.01322662	0.1168866
Pex11a	1419365_at	3.11498759	0.01342737	0.11774318
Wnt5b	1422602_a_a	1.82156613	0.015113	0.12499747
Adrb2	1437302_at	-1.425485	0.01631237	0.13005204
Osbpl11	1436027_at	-1.5133477	0.01644253	0.13044409
Bbs12	1447275_at	2.08525895	0.01676428	0.13158474
Dact1	1417937_at	2.66826005	0.01872908	0.13938951
Asxl1	1458380_at	-1.3363295	0.02003387	0.1445676
Arl4a	1425411_at	1.29146942	0.02051407	0.14648592
Sox8	1435438_at	3.11014067	0.02223232	0.15292445
Zc3h12a	1443993_at	-1.3946394	0.02318622	0.15612903
Zfp385a	1418865_at	0.54201008	0.02368729	0.15748576
Hmga2	1450781_at	1.09586861	0.02694941	0.16831614
Alms1	1456950_at	-0.9174364	0.02699956	0.16844556
Tgfb1i1	1418136_at	2.24534212	0.03175692	0.18296741
Jdp2	1450350_a_a	-1.716068	0.03804747	0.20180716
Crebbp	1459804_at	-1.3274553	0.0394602	0.20599124
Lrg1	1417290_at	-1.3965514	0.04589125	0.22371225
Wnt5a	1448818_at	2.93263031	0.0492736	0.23264251
Adipoq	1422651_at	-0.9677413	0.04950593	0.23318715
Sirt1	1418640_at	0.66458786	0.04979551	0.23372124
Jag1	1434070_at	1.60242889	0.05099174	0.23672447
Arid5b	1458238_at	-0.6856622	0.05148946	0.23793299
Ptgs2	1417263_at	1.37192735	0.0526271	0.24106591
Gata2	1450333_a_a	-0.4200574	0.0567174	0.25105281
Med1	1450402_at	-1.5719065	0.05957002	0.25726603
Dll2	1420807_a_a	0.37367895	0.06840172	0.27672997
Axin1	1426966_at	1.169289	0.06902748	0.27796505
Ppard	1425703_at	0.43412189	0.07675127	0.29440228
Retn	1449182_at	-0.3872131	0.08151515	0.3035349
Cebpa	1418982_at	-0.6341423	0.08246593	0.30538598
Tbl1x	1455042_at	-0.5439523	0.08527682	0.31094428
Cebpb	1418901_at	-0.9722819	0.08544421	0.31125265
Cby1	1451305_at	0.67313195	0.08765196	0.31554846
Mb	1451203_at	-0.5113074	0.08860675	0.31723829
Ncor2	1451841_a_a	0.34038456	0.09045653	0.32098189
Gsk3b	1439931_at	0.83461443	0.09061475	0.32122473
Crebl2	1442738_at	-0.6352672	0.10791559	0.35194886
Aldh6a1	1448104_at	2.58116441	0.10869456	0.35303424
Eif2ak3	1430371_x_a	-1.4225496	0.11979267	0.37163913

Fabp4	1417023_a_a	-1.6804129	0.121138	0.37369664
Ccdc85b	1435589_at	0.27929059	0.12208188	0.37513349
Rarres2	1425091_at	0.31628662	0.12782516	0.38425933
Socs1	1450446_a_a	0.28564968	0.12917782	0.38602244
Sh3pxd2b	1442919_at	0.7836116	0.1378523	0.40031788
Fam57b	1454209_at	0.24715081	0.14388936	0.40878418
Taf8	1416450_at	0.34013535	0.14511102	0.41054822
Lrp6	1451022_at	1.84723464	0.14520466	0.41054822
Runx1t1	1448785_at	0.44754277	0.1596707	0.43041708
Trib2	1426641_at	0.89133272	0.18342065	0.46176136
Ankrd26	1436071_at	0.36205722	0.20661702	0.48819333
Aamdcd	1451381_at	1.06848603	0.21899798	0.50195801
Trib3	1426065_a_a	0.295149	0.23368155	0.51862483
Lpin1	1426516_a_a	0.58391765	0.24135771	0.52699651
Zfp101	1451046_at	-0.1891087	0.25038992	0.53649473
Noc3l	1437500_at	0.78374168	0.25077931	0.53689351
Carm1	1419743_s_a	0.64722551	0.2531193	0.53909773
Ctbp2	1422887_a_a	-0.4047828	0.27076333	0.55490058
Slc2a4	1415959_at	0.17614707	0.27374878	0.55774954
Msx2	1438351_at	0.35290248	0.29922583	0.58127152
Ctbp1	1415702_a_a	0.63070087	0.30707878	0.58884036
Bscl2	1420632_a_a	0.20870757	0.31977026	0.60081479
Socs7	1420766_at	0.74540021	0.33293722	0.61166653
Prdm16	1429309_at	0.14230801	0.3401689	0.61832364
Sfrp1	1428136_at	0.16311129	0.34404904	0.62158781
Mrap	1451371_at	-0.1599235	0.35444922	0.630343
Mettl8	1451141_at	0.66162887	0.3773748	0.6487262
Wnt1	1425377_at	0.36975782	0.37967184	0.65054435
Gm6484	1427422_at	0.12539848	0.40792345	0.67301857
Adrb3	1421555_at	-0.1013874	0.48109302	0.72872465
Aloxe3	1449237_at	0.11167171	0.49007502	0.734659
Sod2	1454976_at	-0.4642386	0.49858924	0.74112024
Sh2b2	1450718_at	0.33787655	0.50093283	0.7428897
Nudt7	1430896_s_a	-0.5009291	0.52179927	0.75602895
Hes1	1418102_at	0.12158584	0.55711349	0.78024953
Gpr116	1440225_at	-0.6471556	0.57903346	0.79420315
Dkk1	1417787_at	0.07057331	0.60962	0.81252932
Uchl1	1448260_at	0.0721505	0.6101289	0.81282636
Cebpd	1456605_at	0.18895067	0.63866748	0.82943279
Gata3	1448886_at	-0.083382	0.65162031	0.83654281
Pparg	1420715_a_a	-0.0670199	0.66484103	0.84418045

Fndc5	1435115_at	0.09998857	0.66491922	0.84418045
Lrp5	1449299_at	0.12006994	0.66717382	0.84534924
Adig	1424729_at	0.05957171	0.67676323	0.8507174
Mmp11	1417234_at	0.08486384	0.71762364	0.87217505
Lep	1422582_at	-0.051409	0.74333518	0.88629937
Sfrp2	1448201_at	0.26766348	0.76534268	0.89810537
Wnt3a	1422093_at	-0.0387946	0.81145358	0.91983231
Adrb1	1423420_at	-0.0217709	0.87321293	0.94746113
Wnt10b	1426091_a_a	0.02676453	0.92652487	0.96924821
Fgf10	1420690_at	-0.0036086	0.98699864	0.99442913

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[0168] All references cited herein are hereby incorporated by reference in their entirety.

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CLAIMS

What is claimed is:

1. A method comprising the steps of:
 - (a) obtaining multipotent mesenchymal stromal cells from a subject, wherein the multipotent mesenchymal stromal cells comprise osteochondroreticular (OCR) stem cells; and
 - (b) isolating from the multipotent mesenchymal stromal cells a population of cells that express Gremlin 1 (*Grem1*) and/or cell surface markers selected from the group consisting of CD200, CD109, and CD105 to produce isolated OCR stem cells.
2. The method of claim 1, further comprising subjecting the multipotent mesenchymal stromal cells to enzymatic digestion.
3. The method of claim 1, wherein the OCR stem cells are isolated via fluorescence-activated cell sorting.
4. The method of claim 1, wherein isolating comprises subjecting mesenchymal stromal cells to enzymatic digestion followed by a CFU assay; and selecting cells comprising highest clonogenicity.
5. The method of claim 1, wherein the multipotent mesenchymal stromal cells are mammalian.
6. The method of claim 5, wherein the multipotent mesenchymal stromal cells are human.
7. The method of claim 1, wherein the isolated OCR stem cells are at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, at least 97% pure, at least 98% pure, at least 99% pure, at least 99.5% pure, or at least 99.9% pure osteochondroreticular (OCR) stem cells.
8. A method comprising subjecting isolated OCR stem cells to conditions that promote differentiation into osteoblasts, chondrocytes, and reticular marrow stromal cells.
9. The method of claim 8, wherein the conditions that promote differentiation comprise culturing the OCR stem cells in the presence of differentiation medium, the medium comprising a bone morphogenic protein.

10. A composition comprising an acceptable carrier and isolated OCR stem cells that express at least the cell marker *Grem 1*.
11. A method of treating disease, degeneration, or injury of bone, or cartilage, or both, in a subject comprising administering a therapeutically effective amount of a composition of claim 10 to a site in need in the subject.
12. The method of claim 11 wherein the site of need is a joint and the therapeutically effective amount of the composition is administered into a space of the joint or into articular tissue of the joint.
13. The method of claim 12, wherein the disease treated is osteoarthritis.
14. The method of claim 11, wherein the site of need is a fracture, and the therapeutically effective amount of the composition is administered into the fracture or tissue surrounding the fracture, or both.
15. A kit comprising a container in which the composition of claim 10 is contained.
16. The kit of claim 15, wherein the container is a vial, tube, syringe or bag.
17. A method comprising
 - applying a sample of OCR stem cells to a biocompatible scaffold to produce a cell-seeded scaffold; and
 - subjecting the cell seeded scaffold to cell culture conditions for a sufficient amount of time to allow the OCR stem cells to divide and populate the biocompatible scaffold to produce an implant.
18. The method of claim 17, wherein the cell culture conditions comprise incubating the seeded scaffold in cell culture media comprising one or more factors that promote differentiation of the OCR stem cells into osteoblasts, chondrocytes, or reticular marrow stromal cells.
19. An implant produced by the method of claim 17.

20. A method of treating osteoarthritis of a joint comprising administering a therapeutically effective amount of OCR stem cells to the joint.

21. A method comprising

obtaining a population of intestinal reticular stem cells (iRSCs); and

administering the population of iRSCs into an intestine of a subject in need thereof, wherein the administered population generates periepithelial mesenchymal sheath.

22. The method of claim 21, wherein the population is produced by isolating cells from an intestinal tissue sample that express Gremlin 1 (Grem1) and subjecting the isolated cells to form a clone.

23. The method of claim 21, wherein the population of cells are subjected to conditions to form a gut organoid unit prior to administration.

24. A method comprising the steps of:

(a) obtaining an intestinal cell sample from a subject, wherein the intestinal cell sample comprises intestinal reticular stem cells (iRSCs); and

(b) isolating from the cell sample a population of cells that express Gremlin 1 (*Grem1*), to produce a sample of isolated iRSCs.

25. The method of claim 24, wherein the isolated iRSCs are subjected to culture conditions to produce a gut organoid.

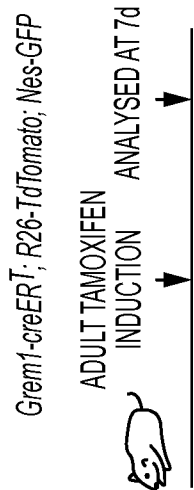


FIG. 1A

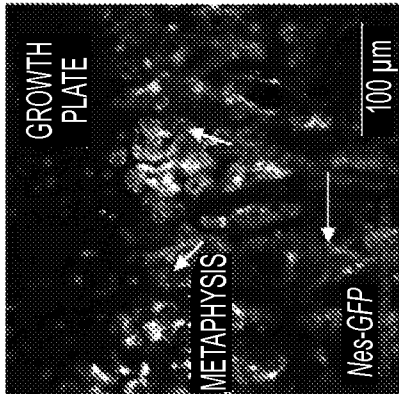
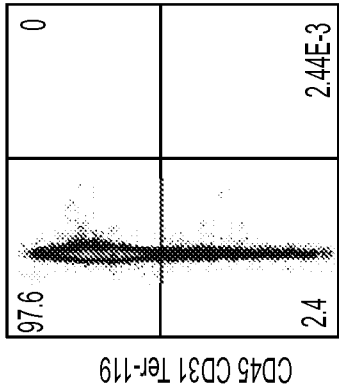
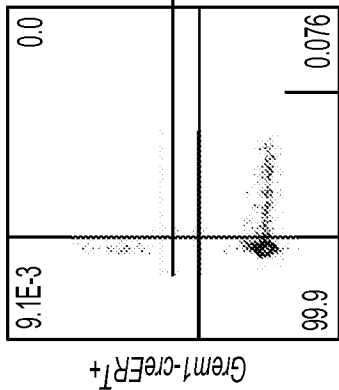


FIG. 1B



Grem1-creERT⁺

FIG. 1C



Nes-GFP

FIG. 1D

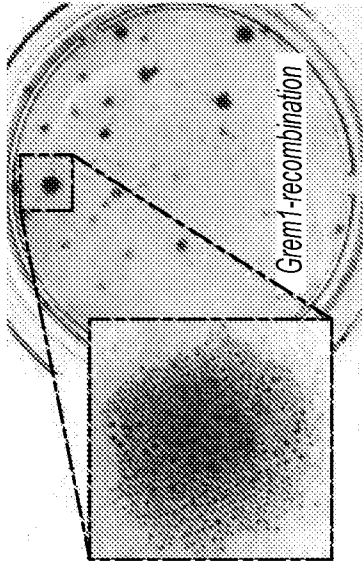


FIG. 1E

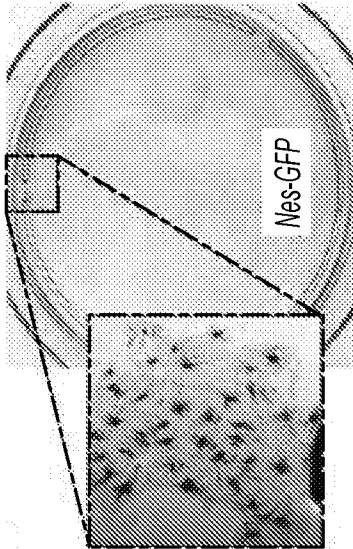


FIG. 1F

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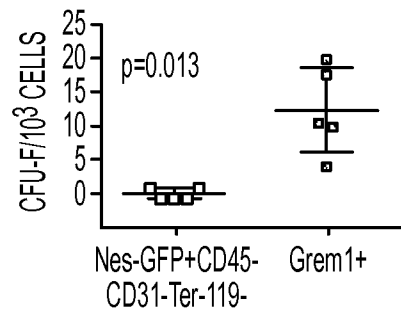


FIG. 1G

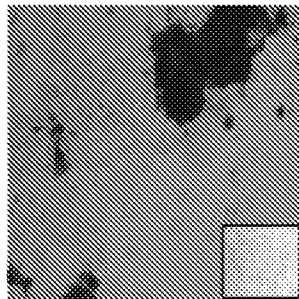


FIG. 1H

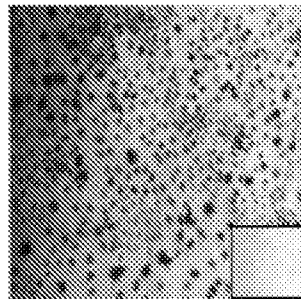


FIG. 11

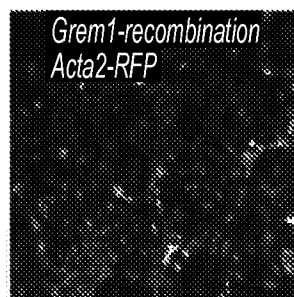


FIG. 1J

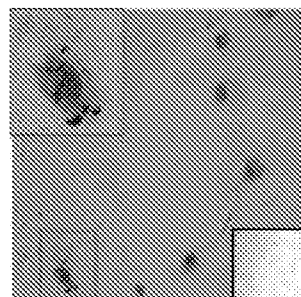


FIG. 1K

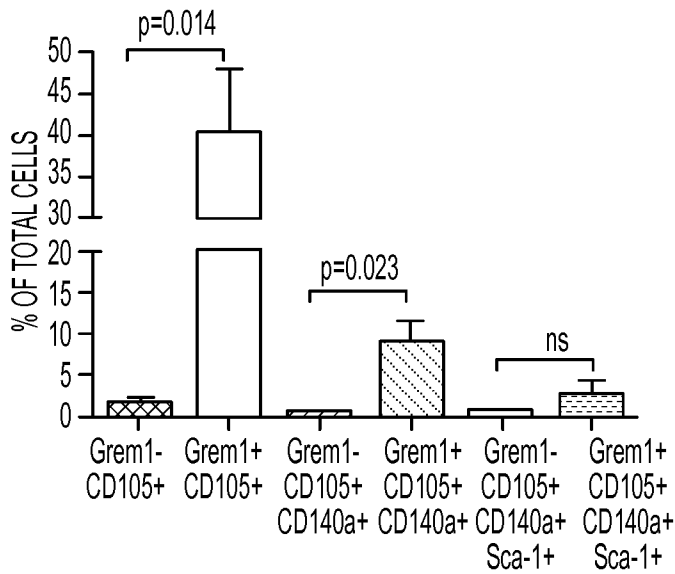
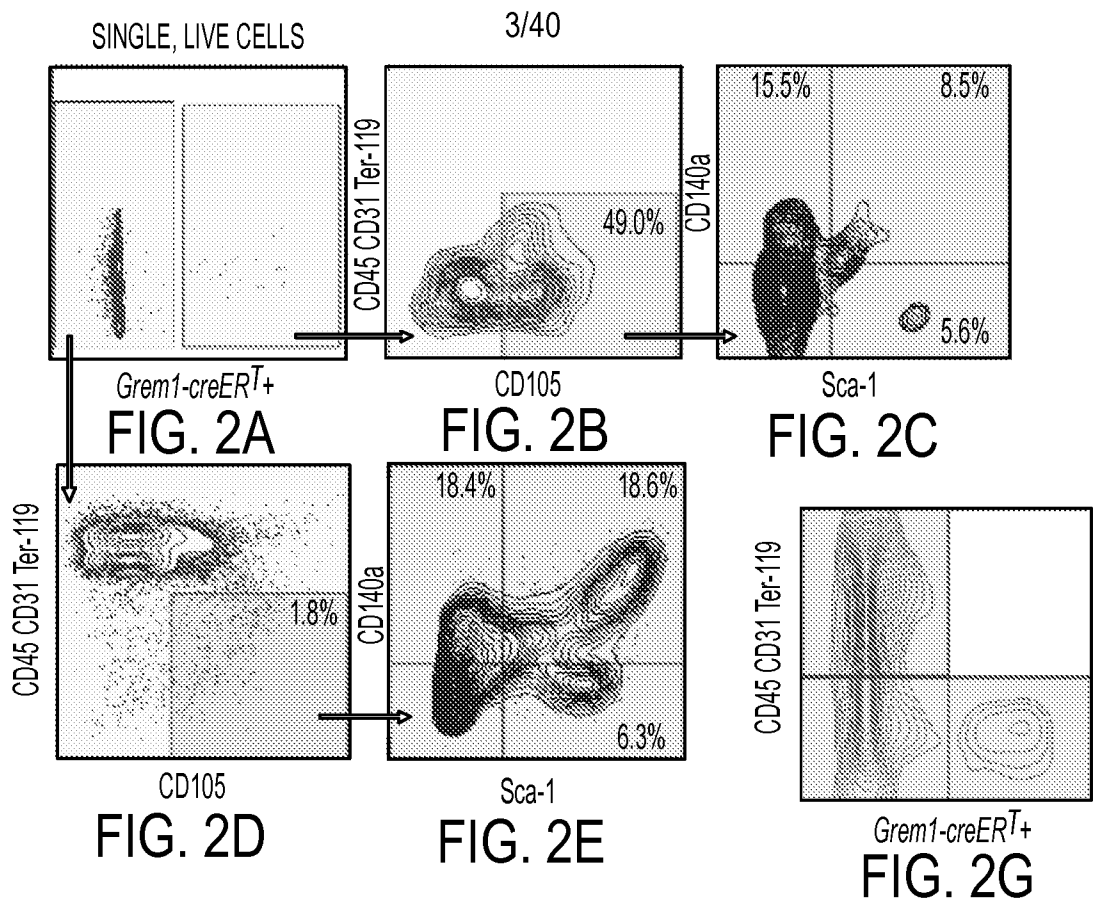


FIG. 2F

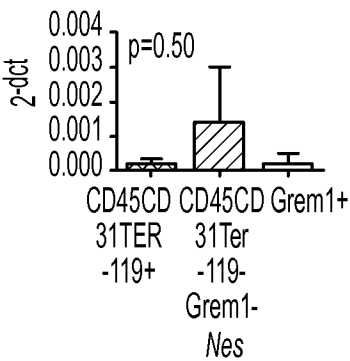


FIG. 2H

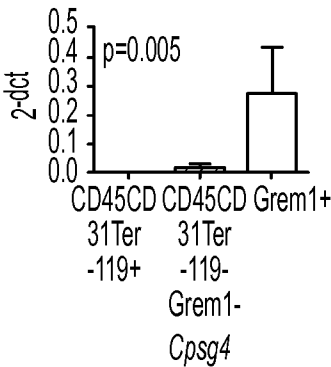
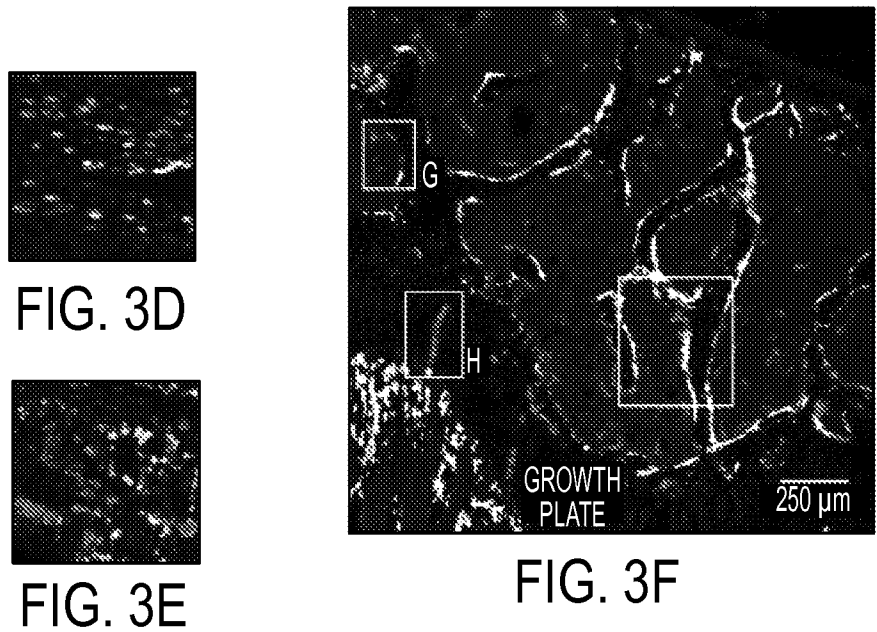
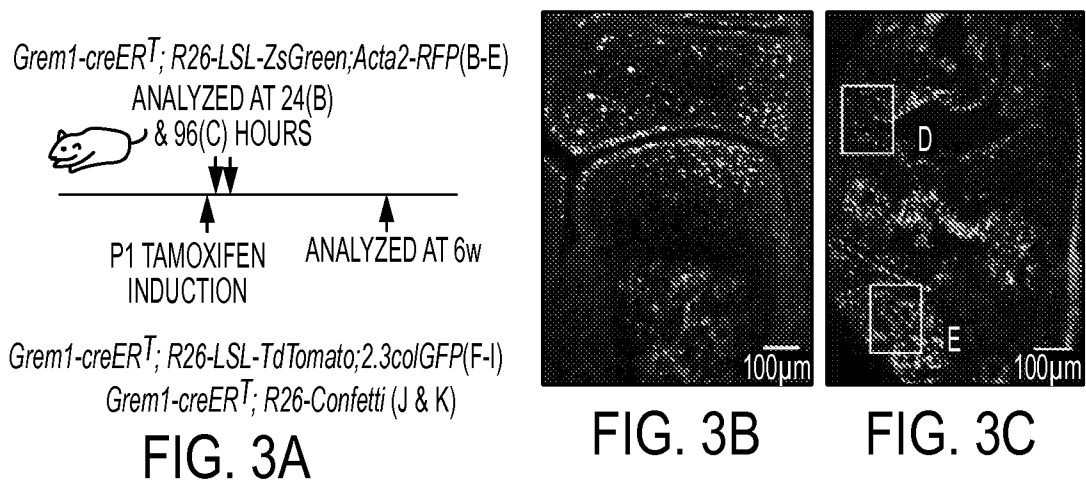
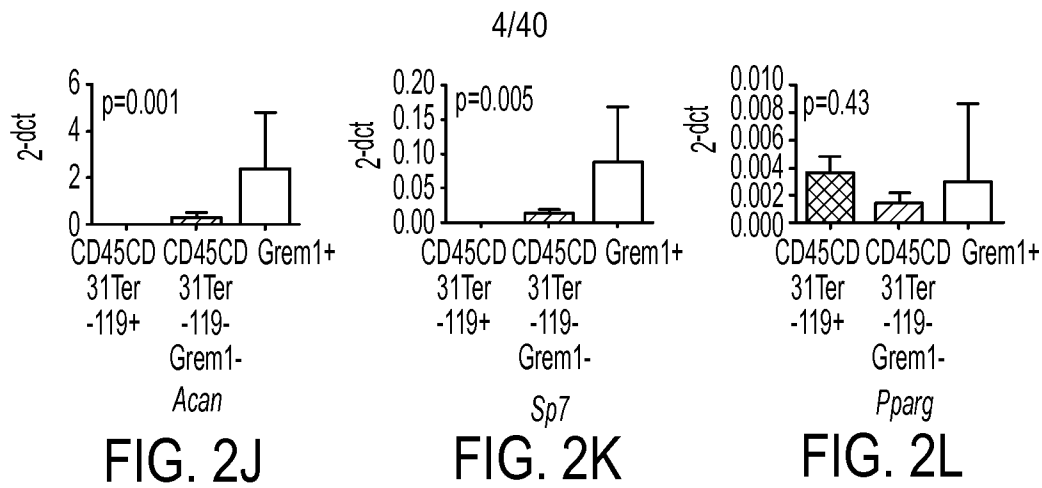


FIG. 2I



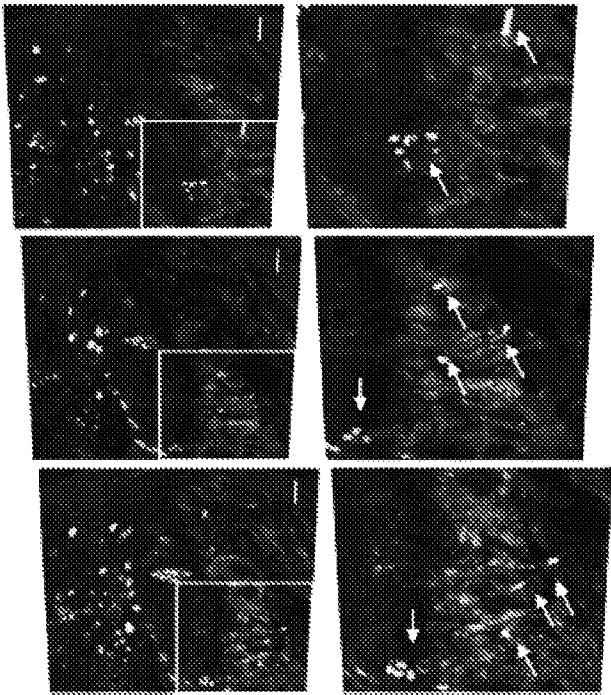


FIG. 3K

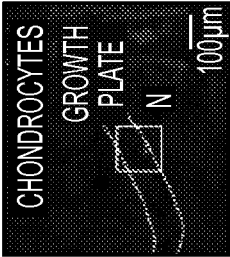


FIG. 3M

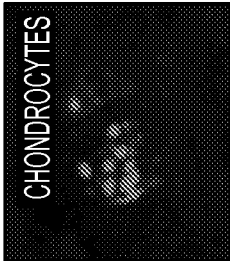


FIG. 3N



FIG. 3O



FIG. 3G

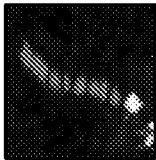


FIG. 3H



FIG. 3I

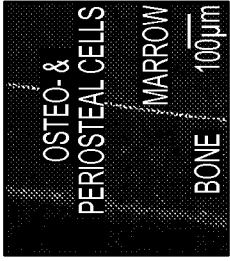


FIG. 3P

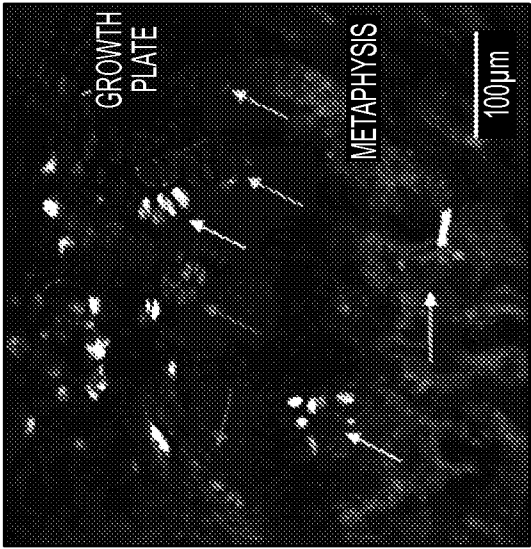


FIG. 3J



FIG. 3L

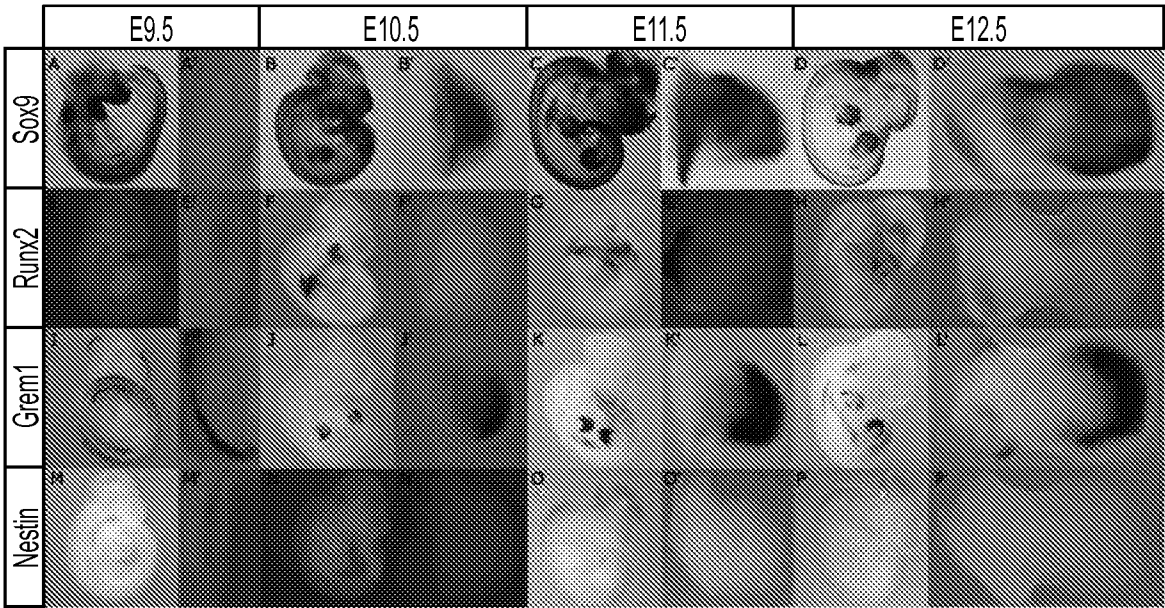


FIG. 4A

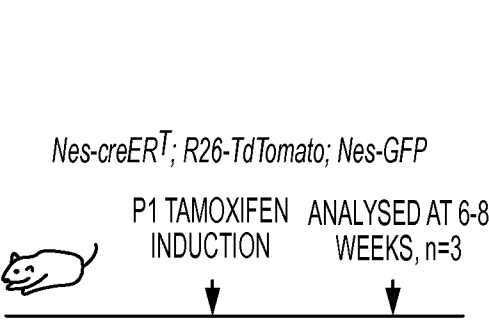


FIG. 4B

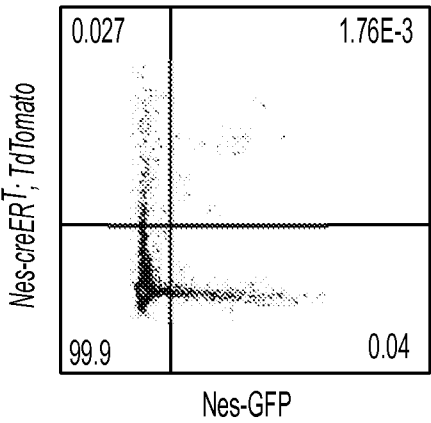


FIG. 4C

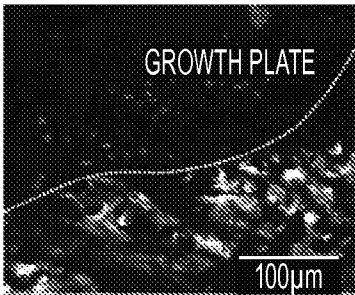


FIG. 4D

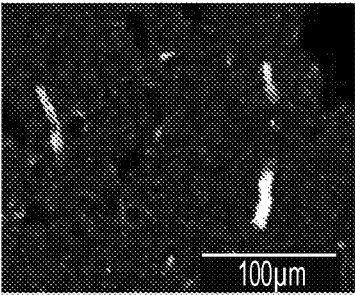


FIG. 4E

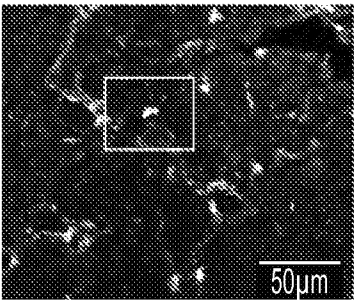


FIG. 4F

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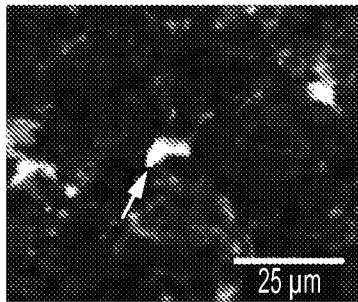


FIG. 4G

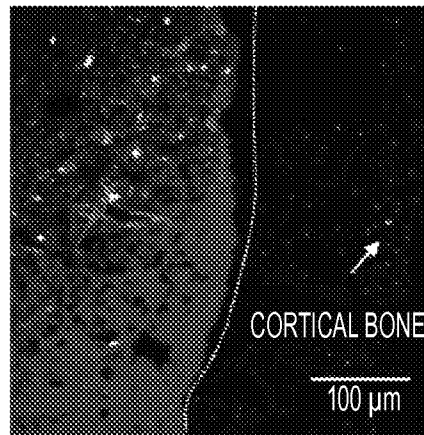


FIG. 4H

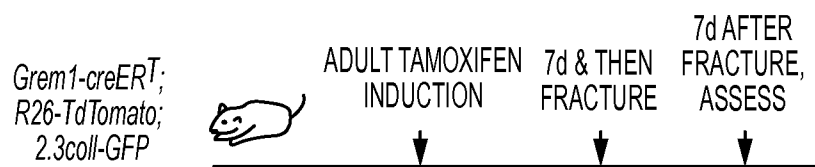


FIG. 5A

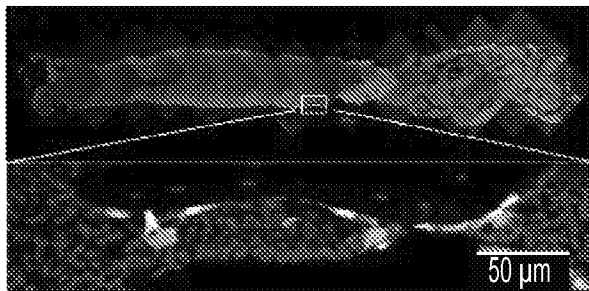


FIG. 5B

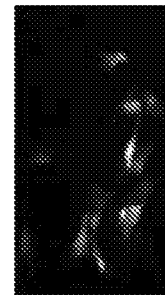


FIG. 5C

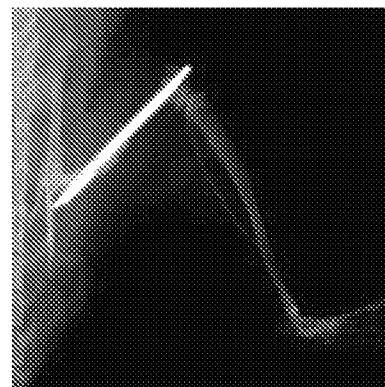


FIG. 5D

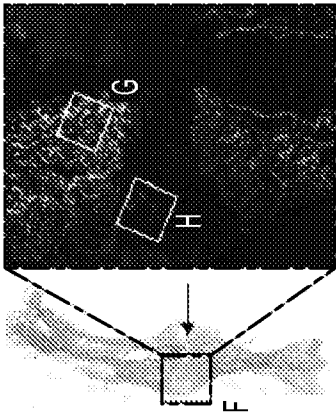


FIG. 5E



FIG. 5F

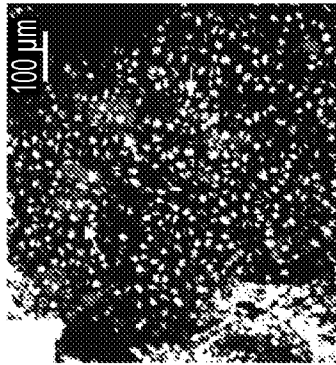


FIG. 5G



FIG. 5H

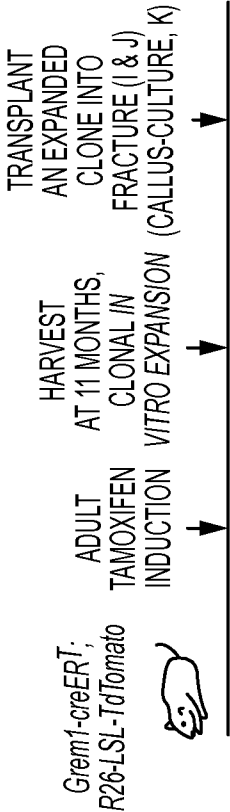


FIG. 5I

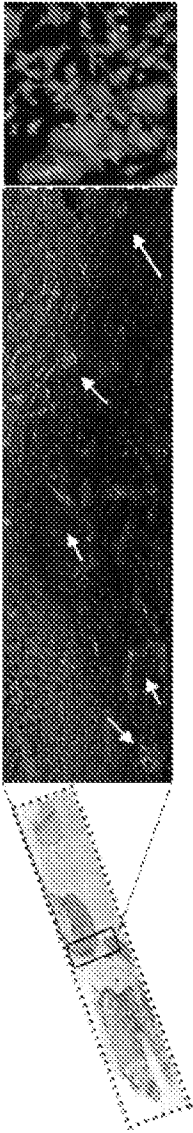


FIG. 5J

FIG. 5K

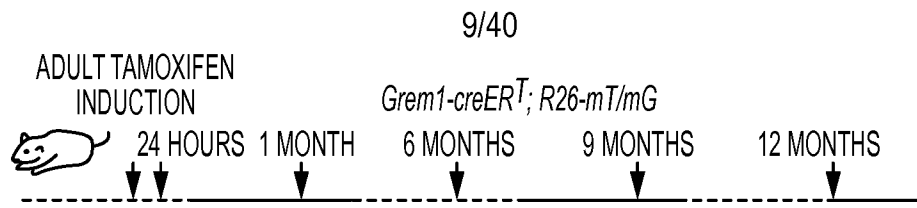


FIG. 6A

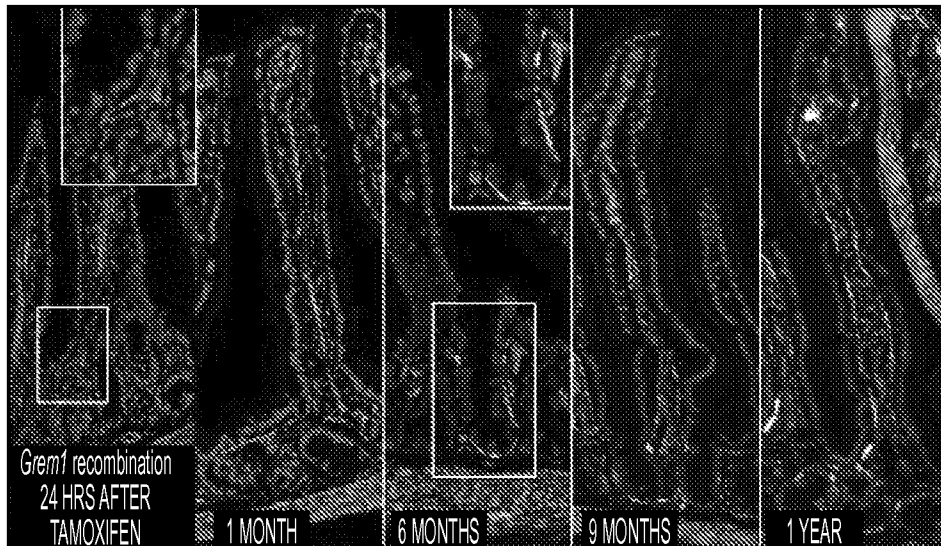


FIG. 6B

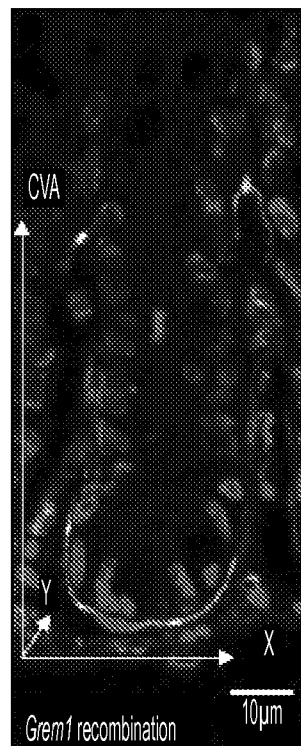


FIG. 6C

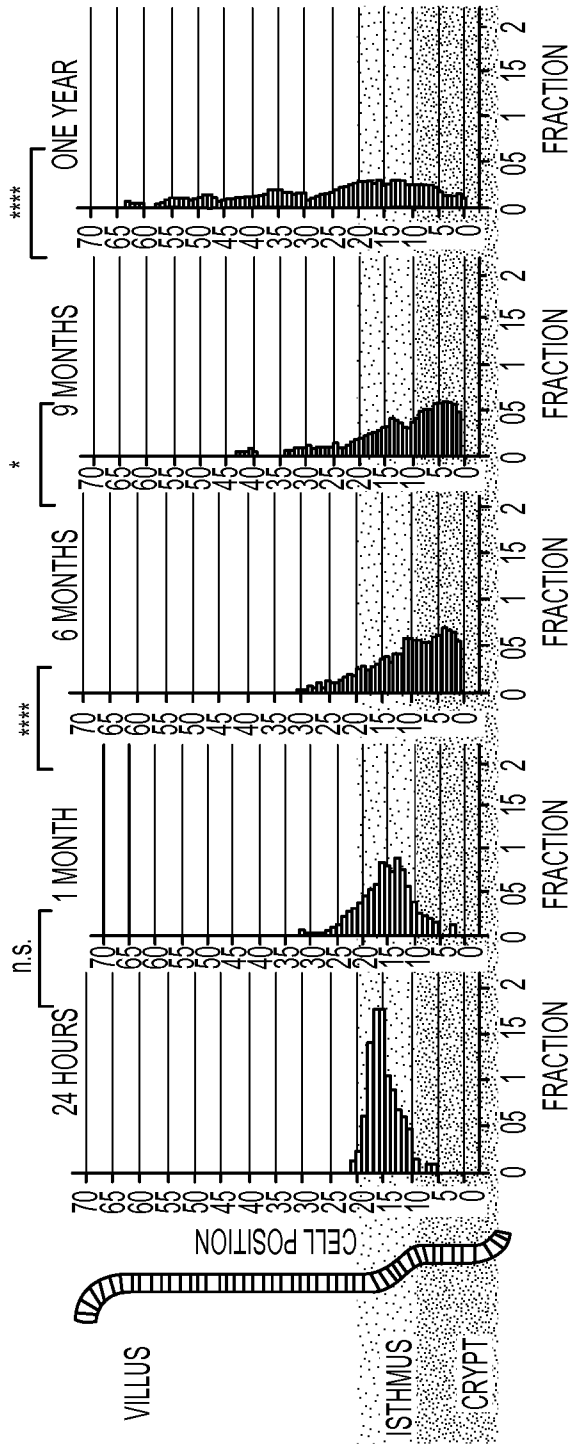


FIG. 6D

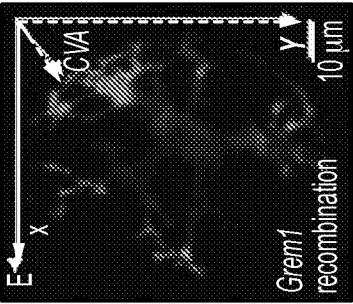


FIG. 6E

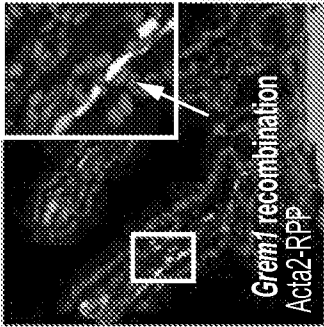


FIG. 6F

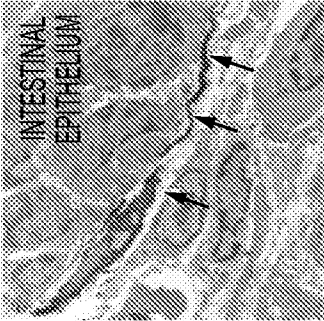


FIG. 6G



FIG. 6H

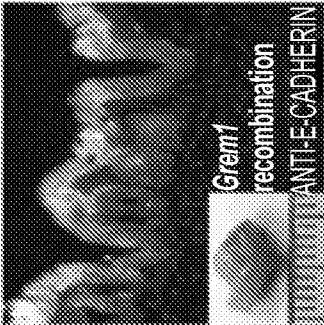


FIG. 6I

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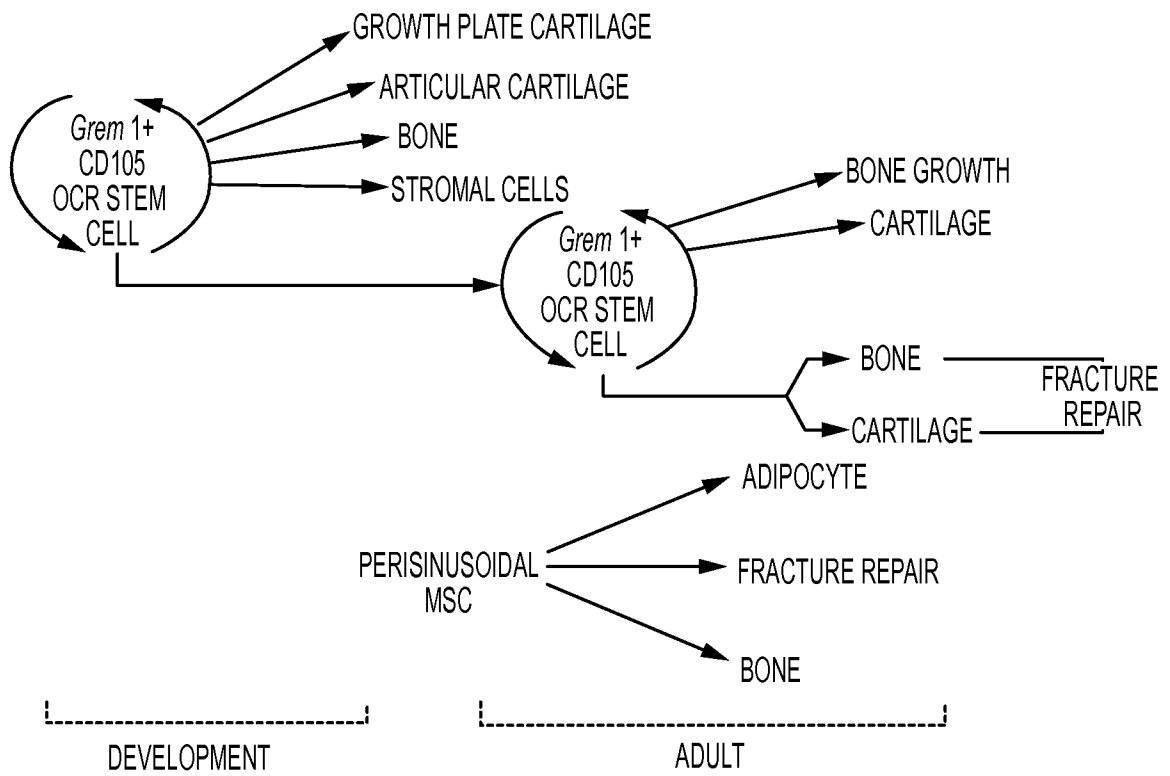


FIG. 7

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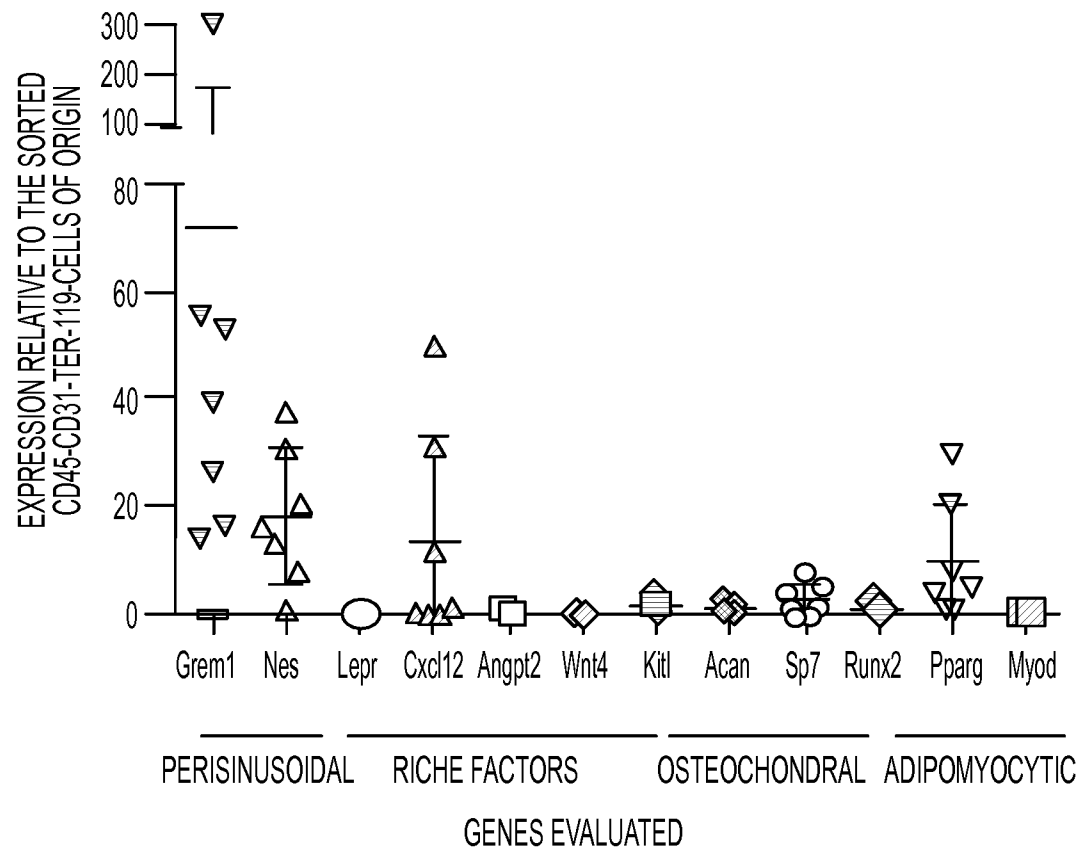


FIG. 8A

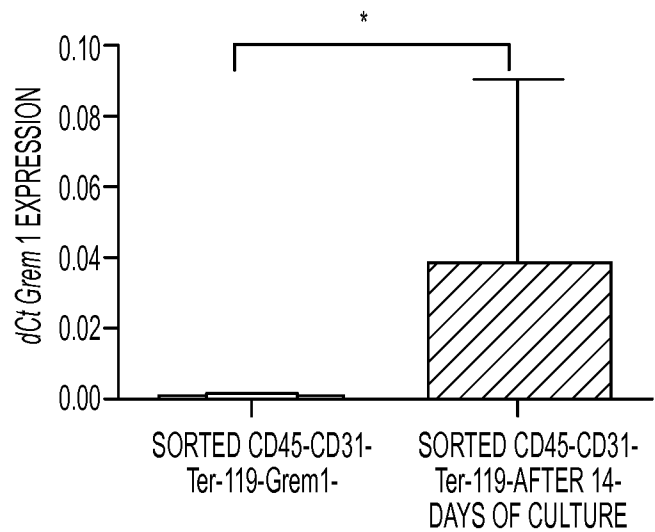


FIG. 8B

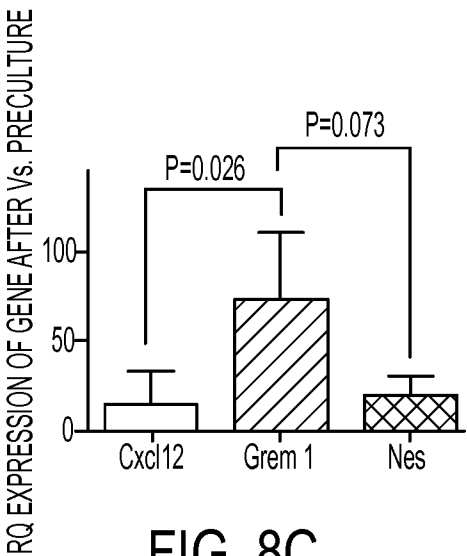
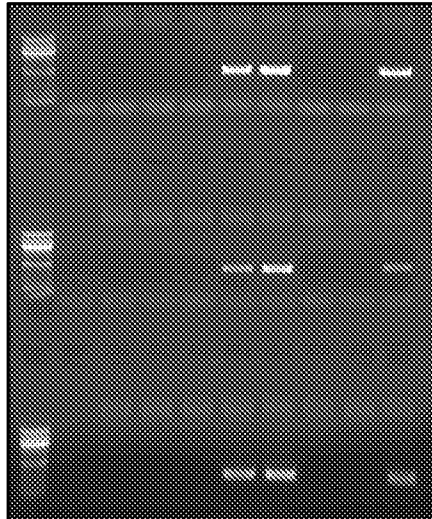


FIG. 8C

13/40

100 bp
LADDER

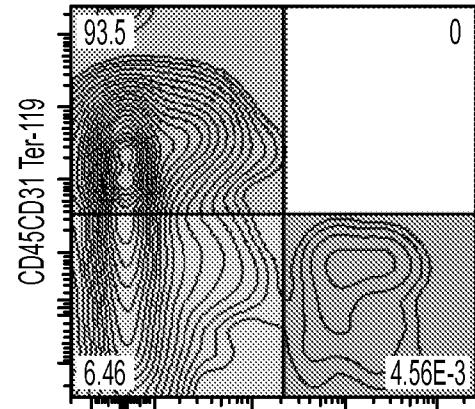
NEGATIVE CONTROL 8 PUPS, 3 POSITIVE
LINE 1 2 3



5' INSERT PCR

3' INSERT PCR

Cre PCR



Grem1-TdTomato

FIG. 8D

FIG. 8E

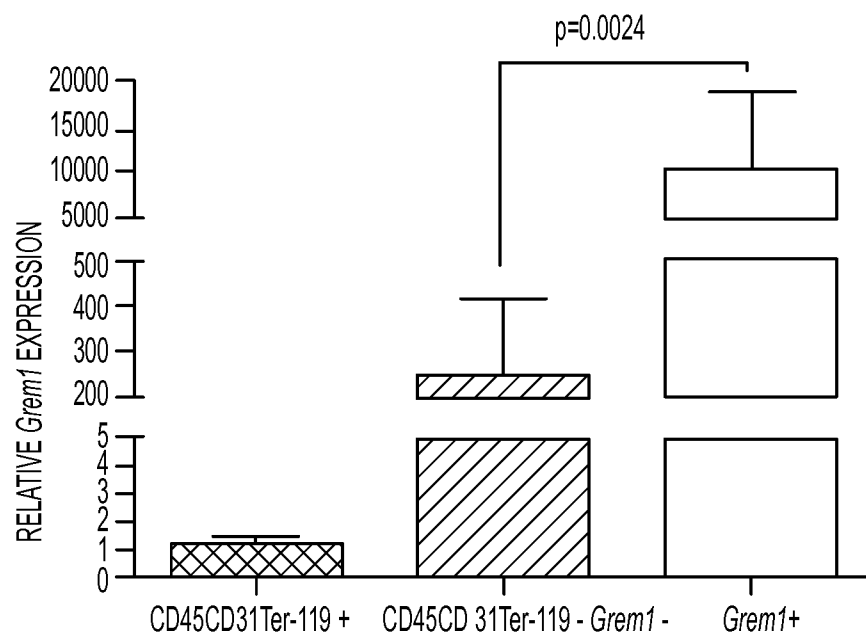


FIG. 8F

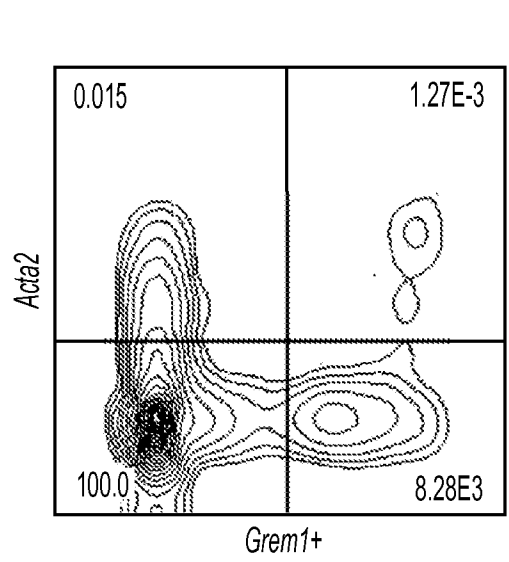


FIG. 9A

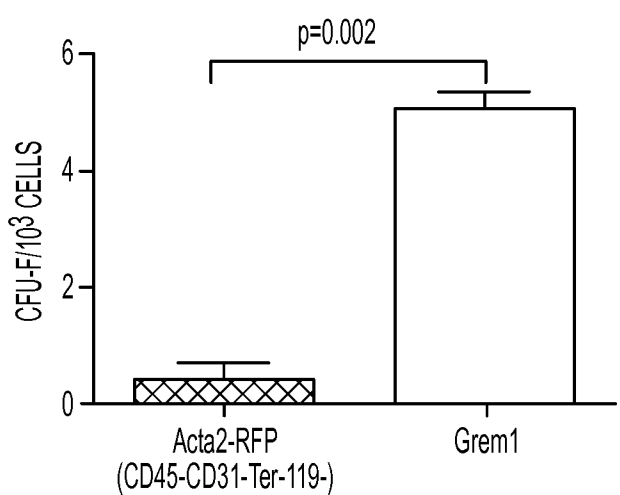


FIG. 9B

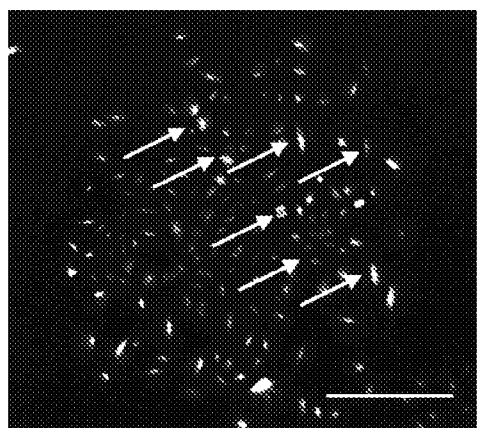


FIG. 9C

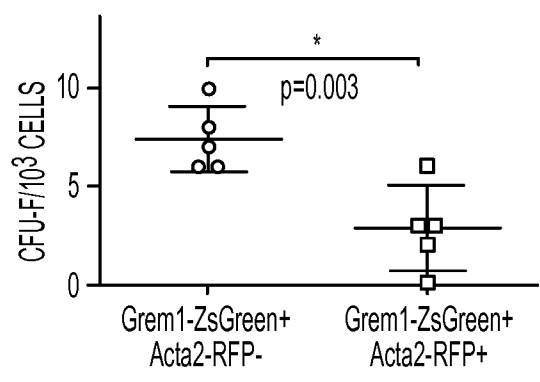


FIG. 9D

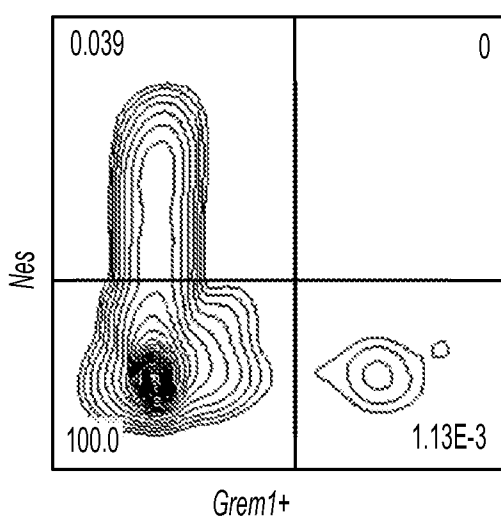


FIG. 9E

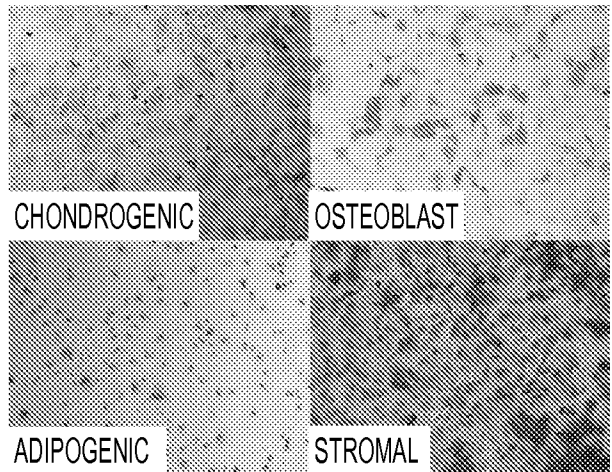
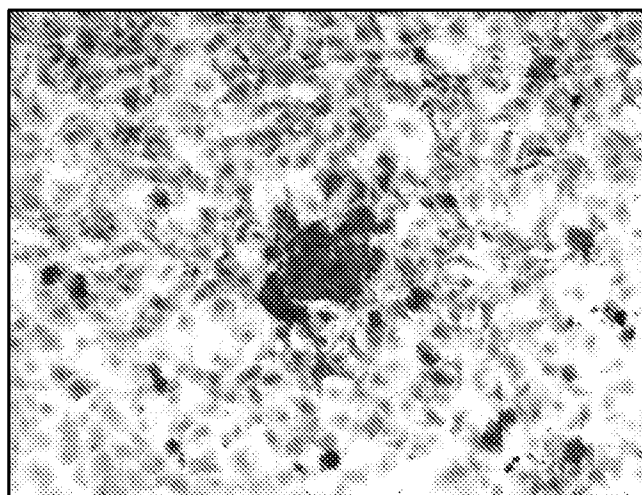


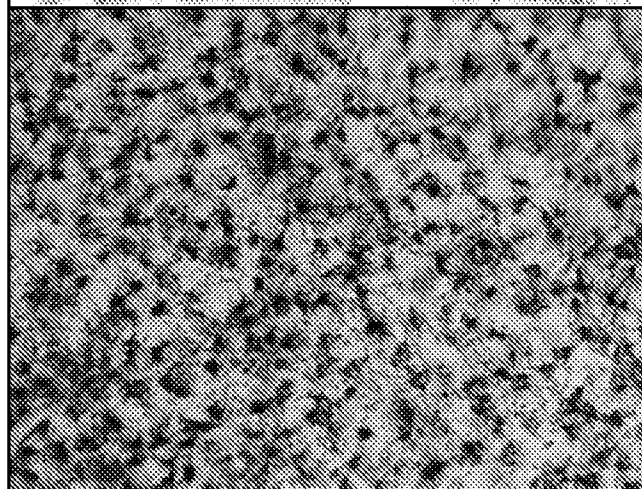
FIG. 9F

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ALIZARIN RED



ALCIAN BLUE



OIL RED



FIG. 9G

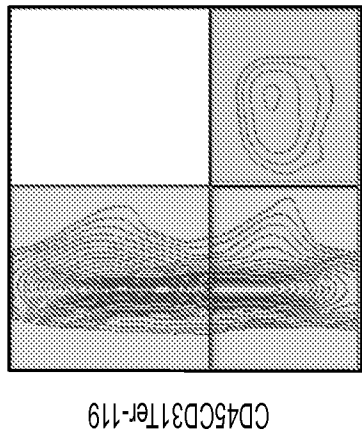


FIG. 10A

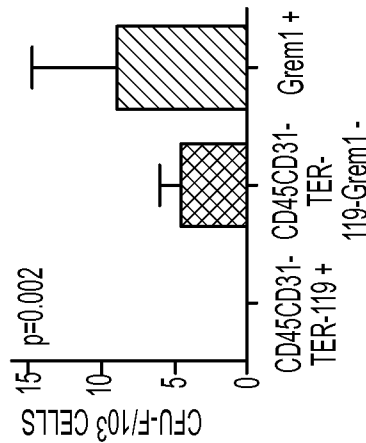


FIG. 10B

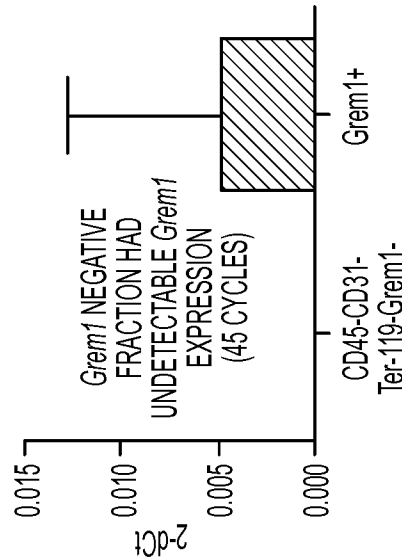


FIG. 10C

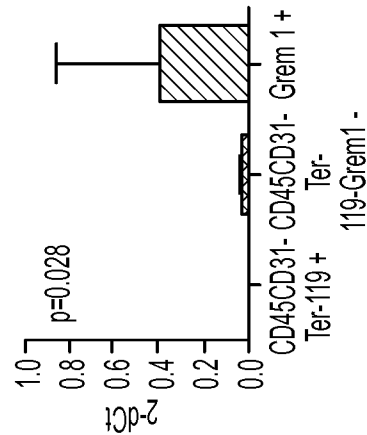


FIG. 10D

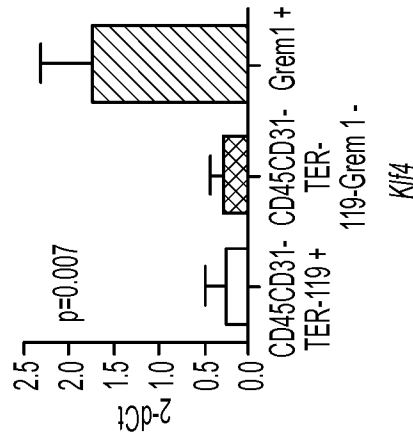


FIG. 10E

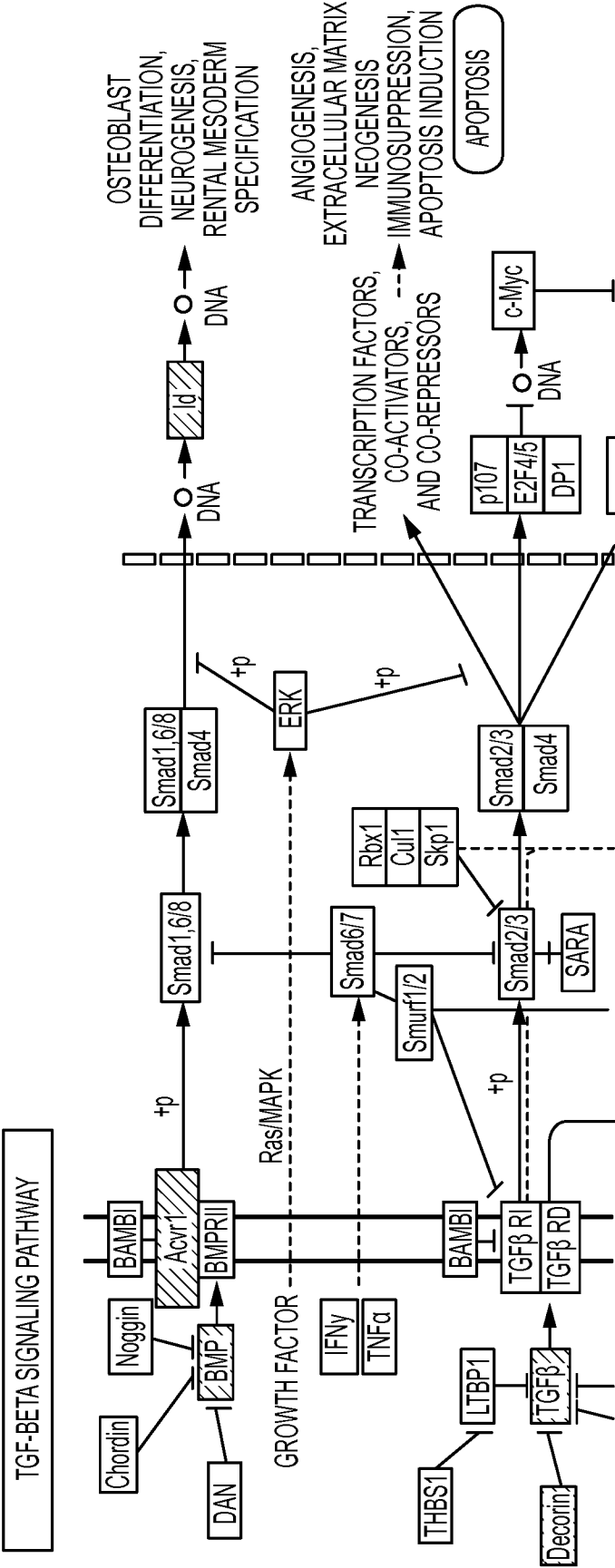


FIG. 10F

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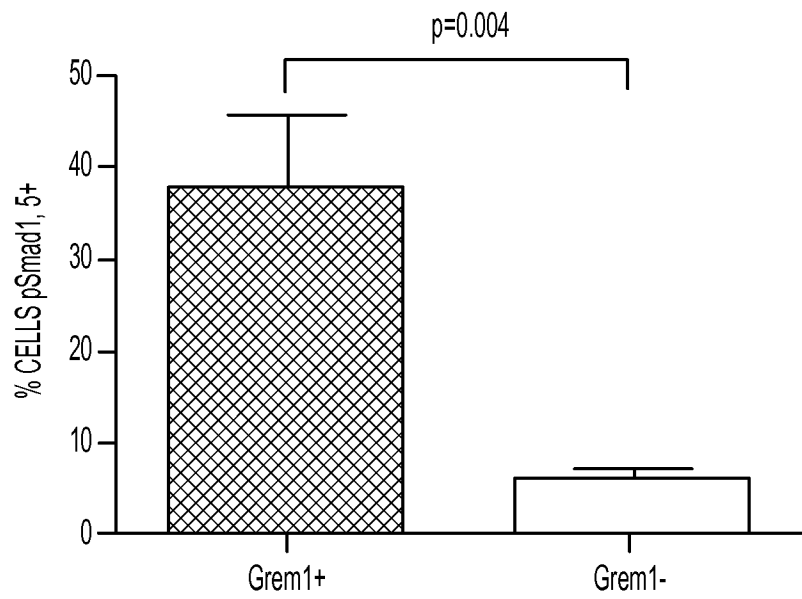


FIG. 10G

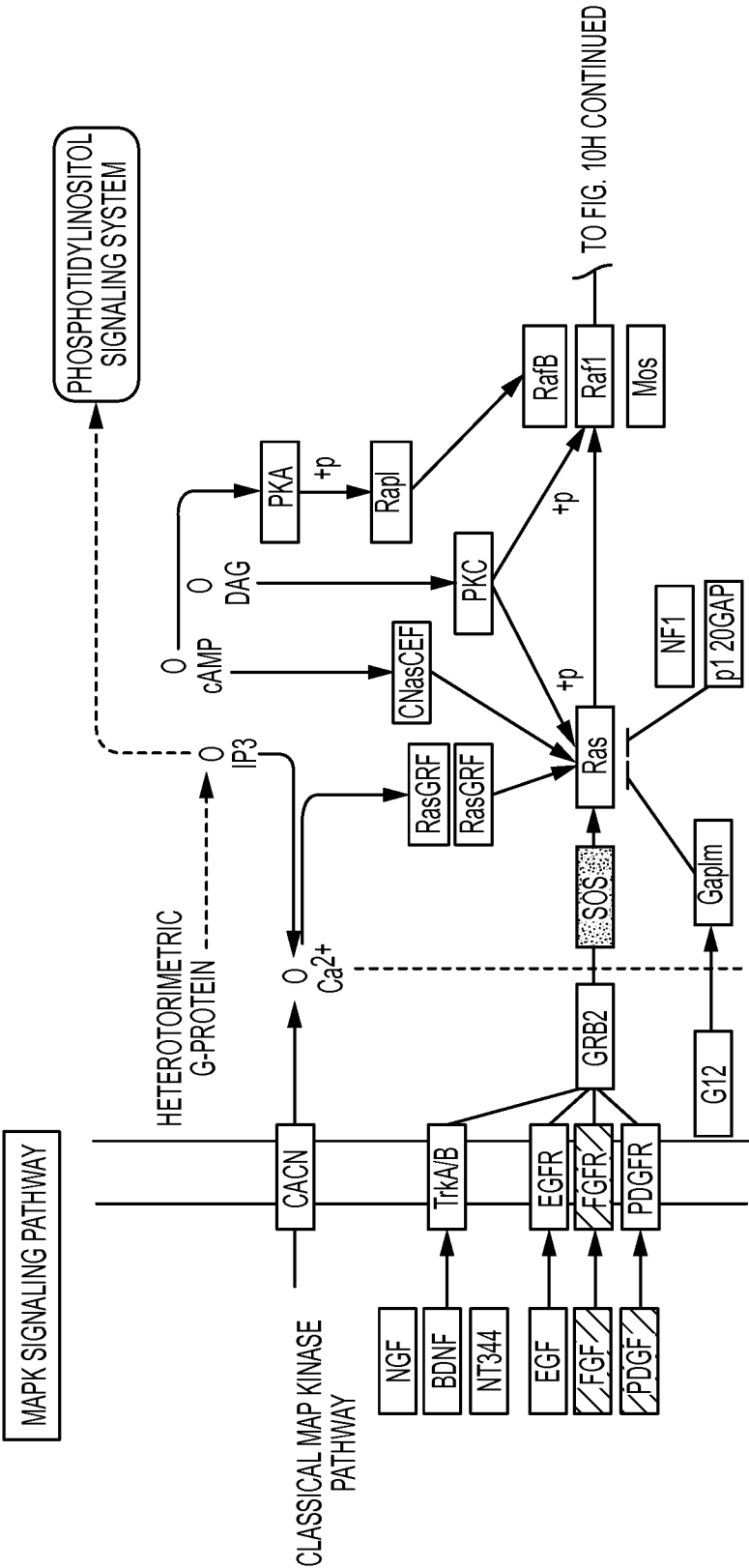


FIG. 10H

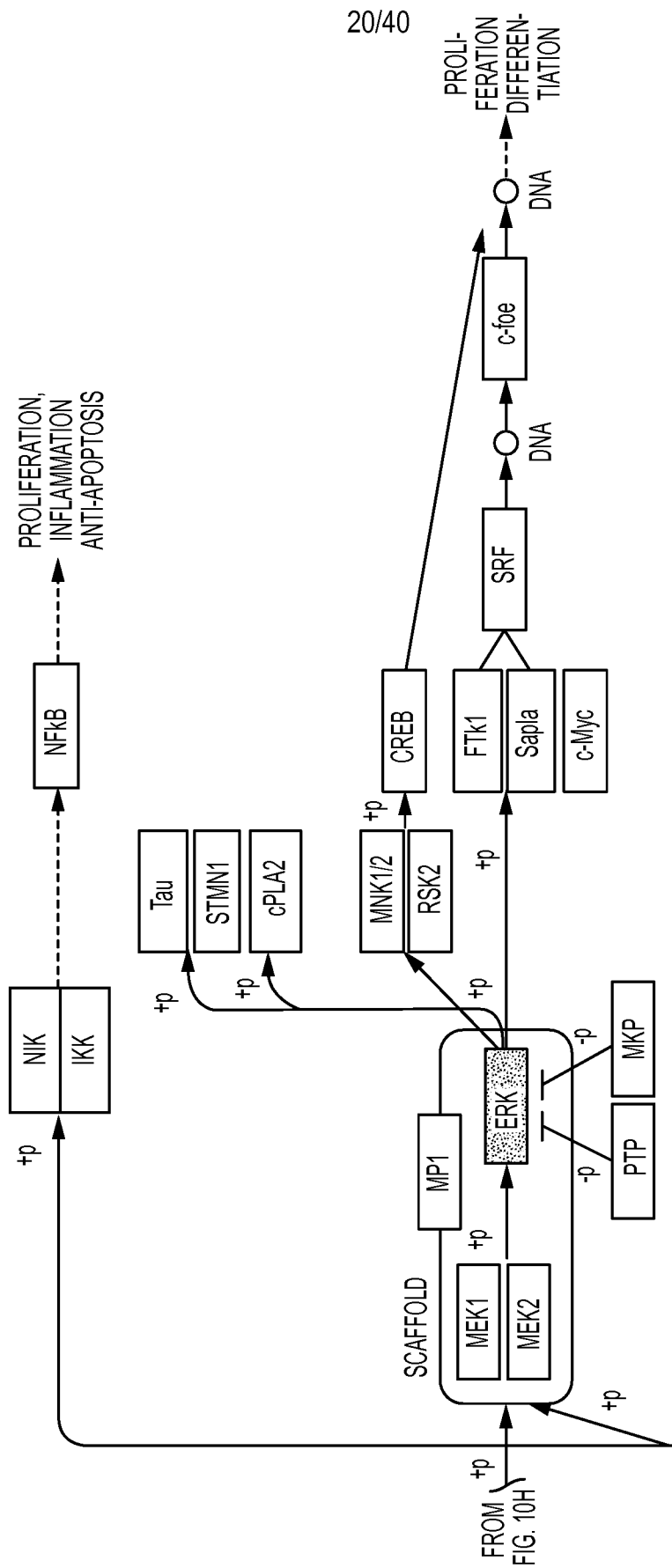


FIG. 10H
CONTINUED

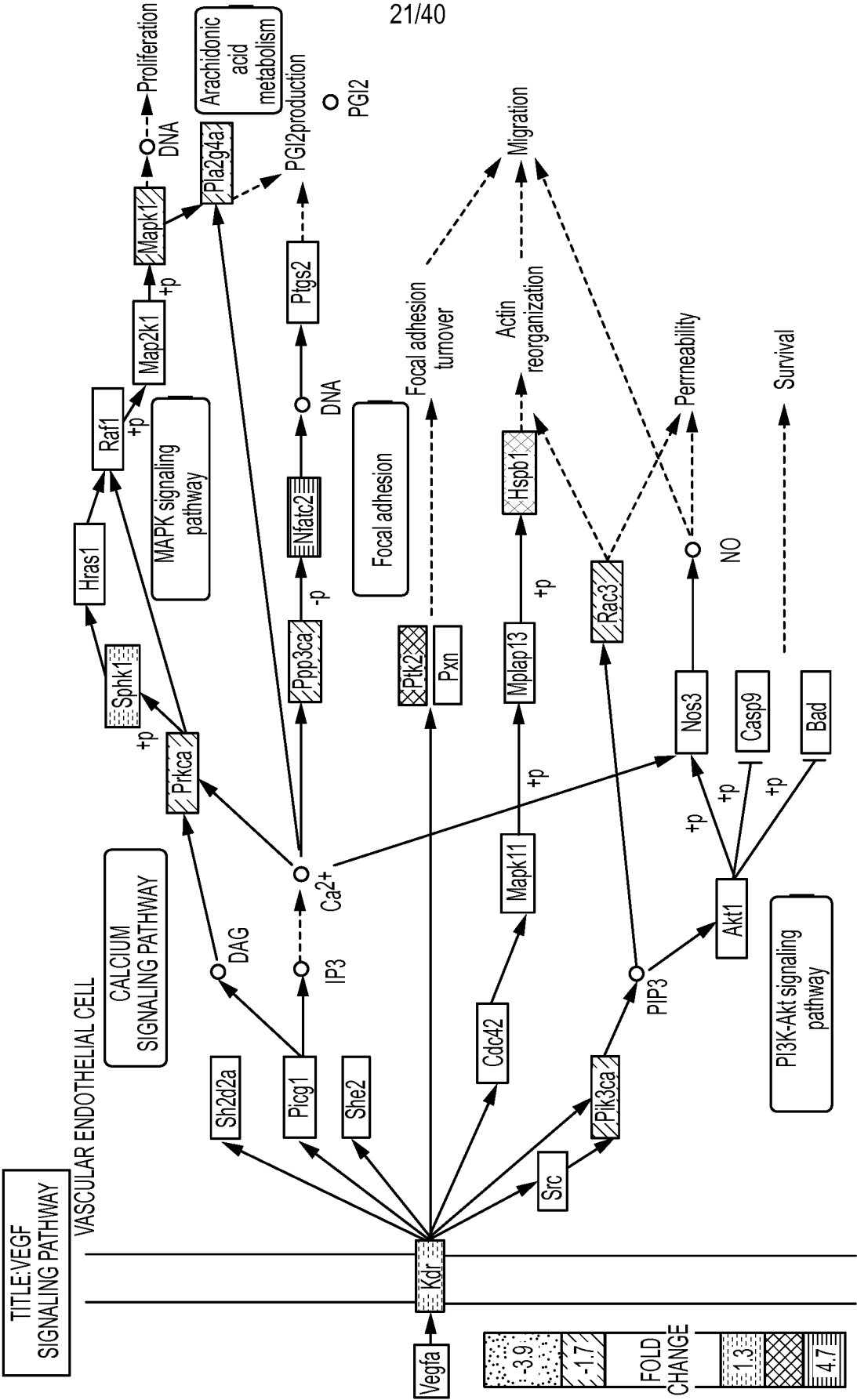


FIG. 10I

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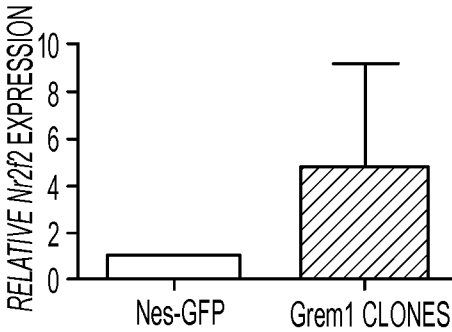


FIG. 10J

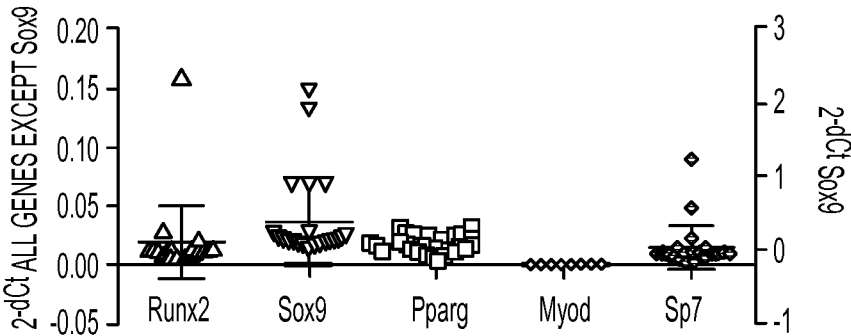


FIG. 10K

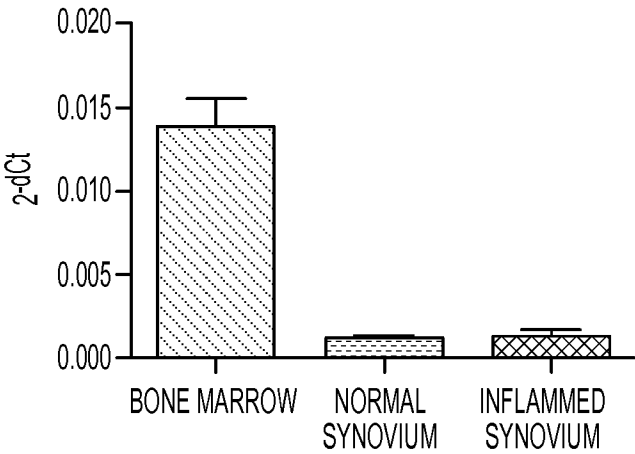


FIG. 10L

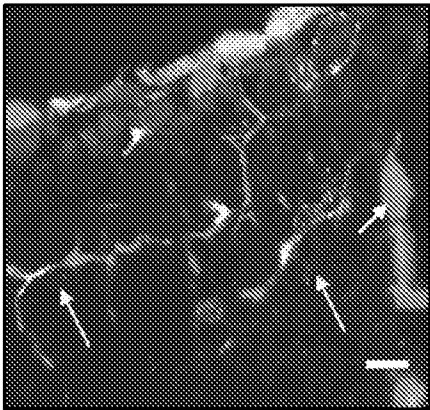


FIG. 11A

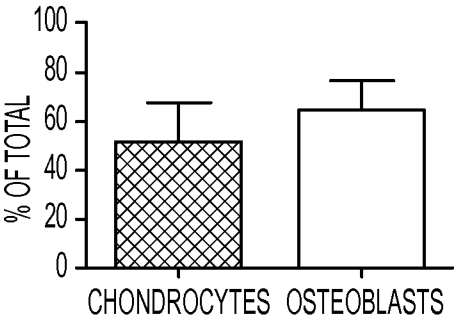


FIG. 11B

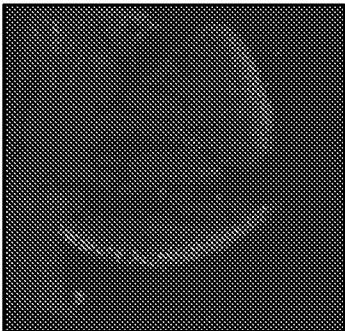


FIG. 11C

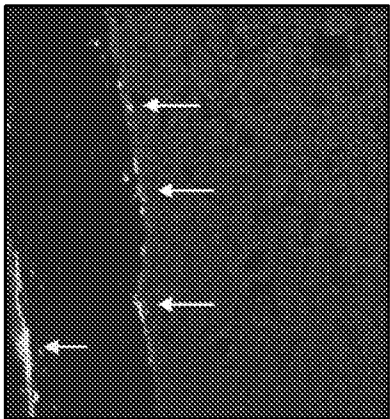


FIG. 11D

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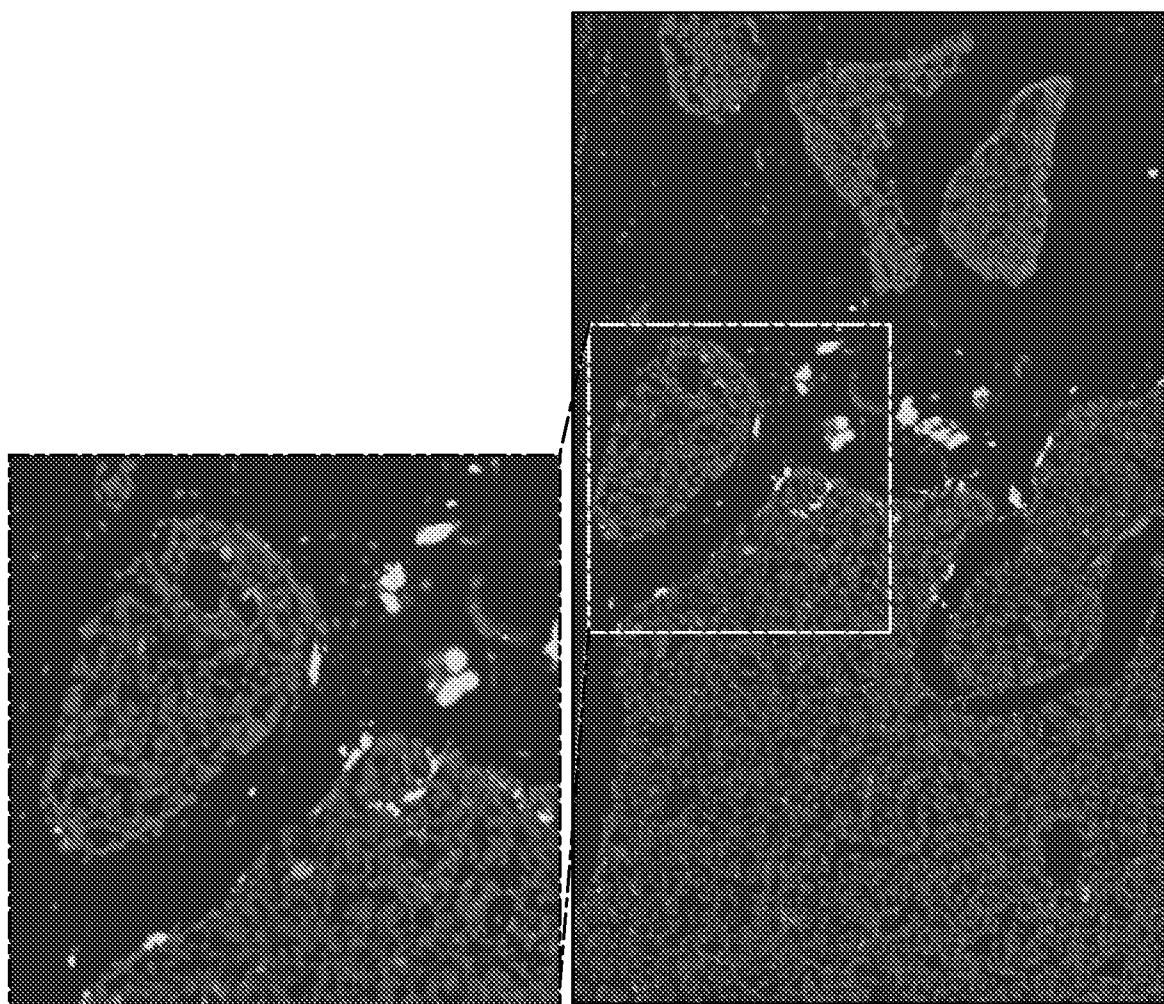


FIG. 11E

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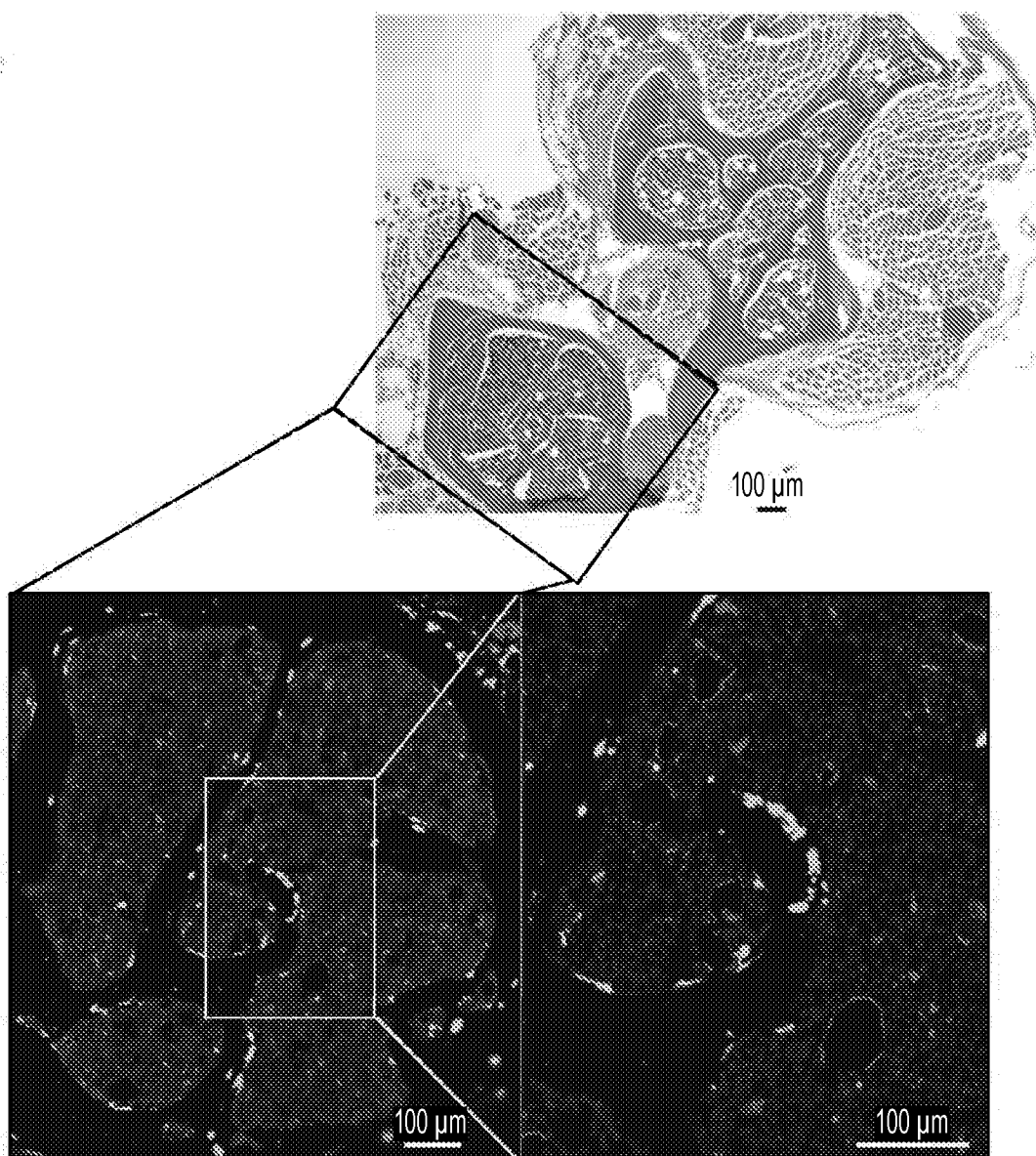


FIG. 11F

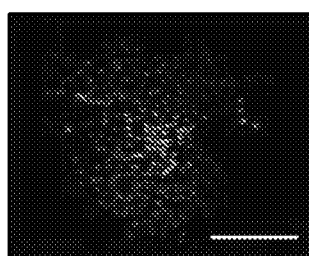


FIG. 11G

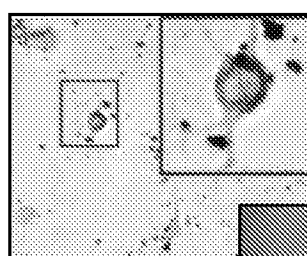


FIG. 11H

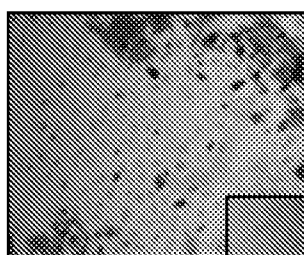


FIG. 11I

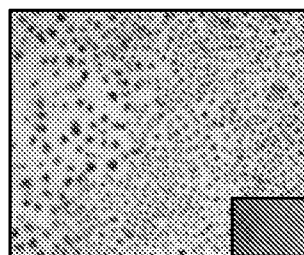


FIG. 11J

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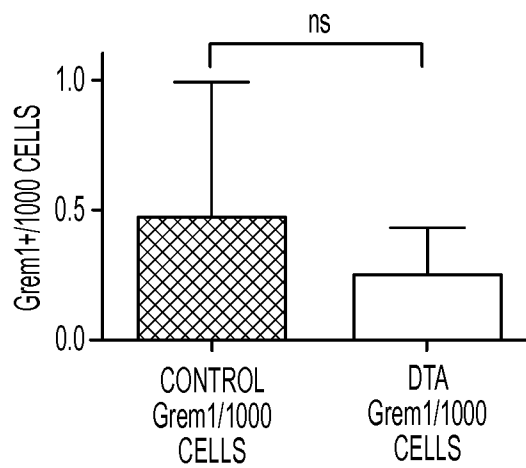


FIG. 12A

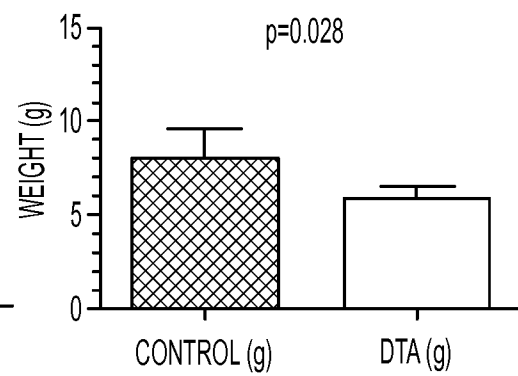


FIG. 12B

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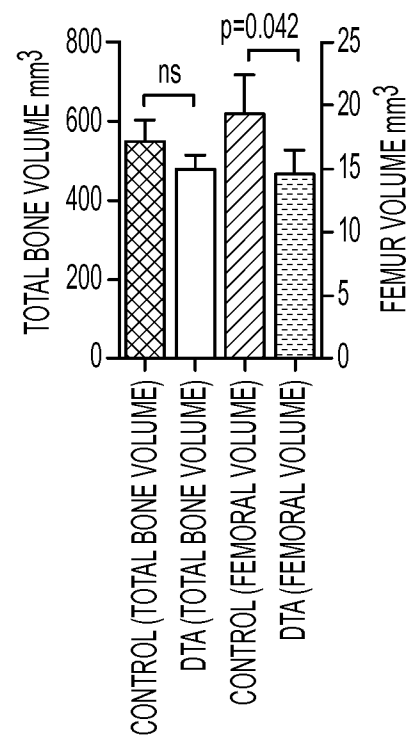
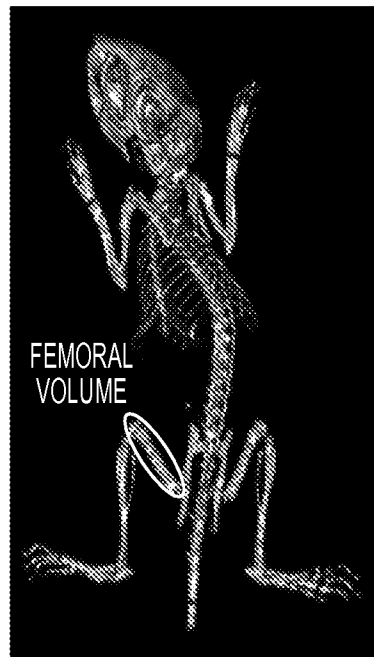
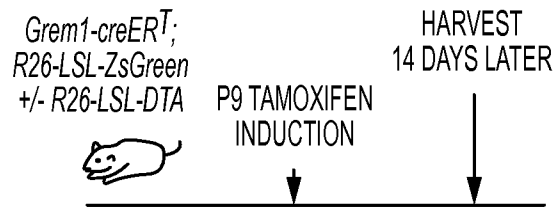


FIG. 12C

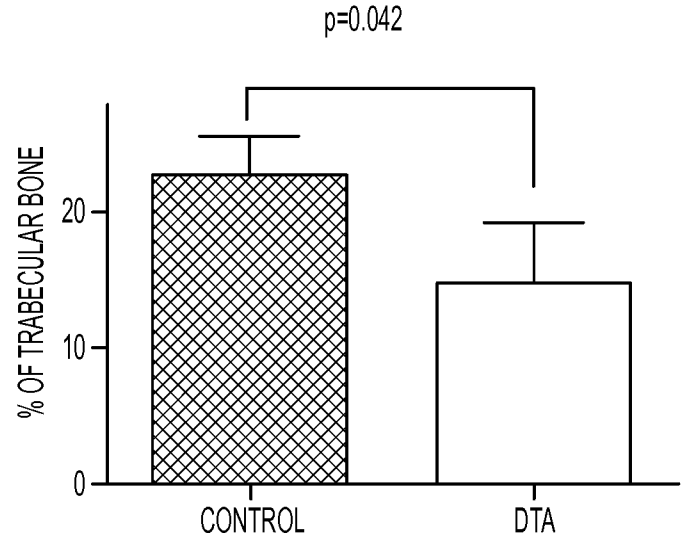
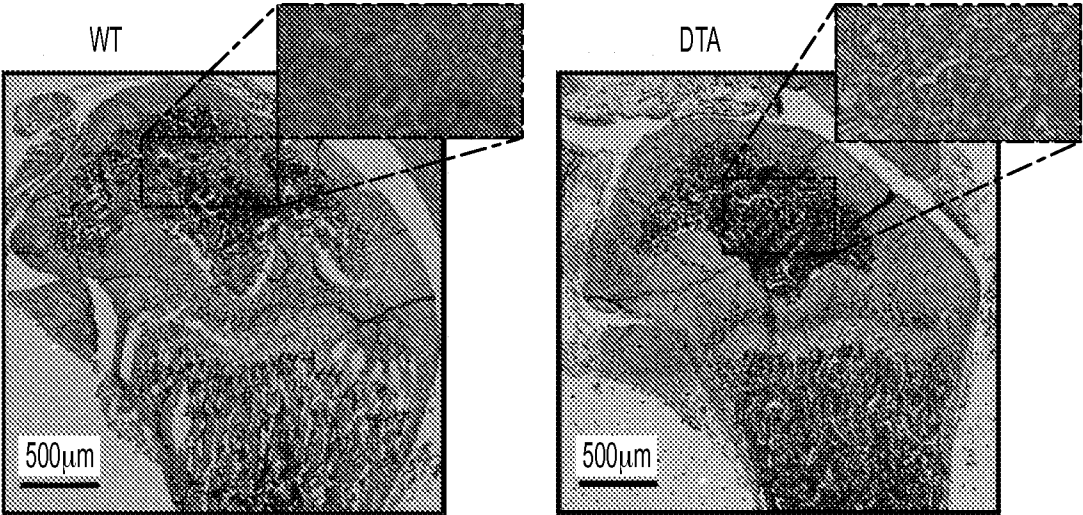


FIG. 12D

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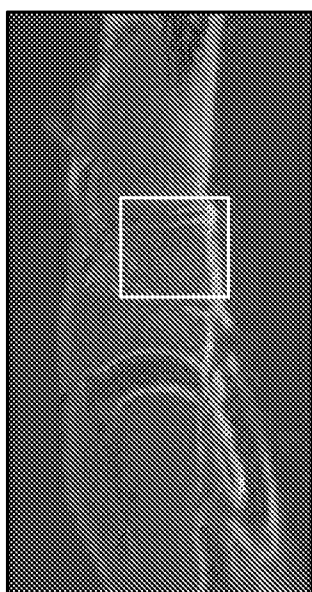


FIG. 12E

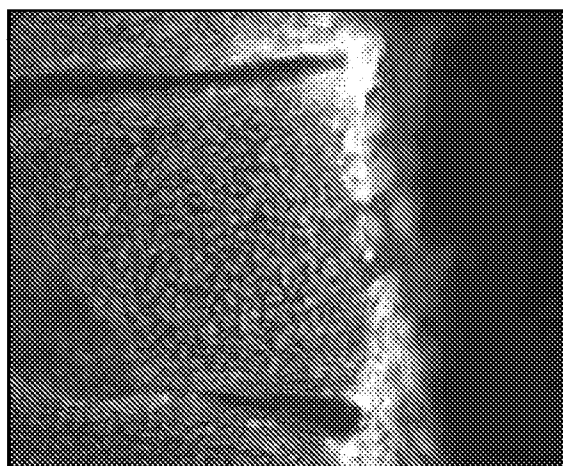
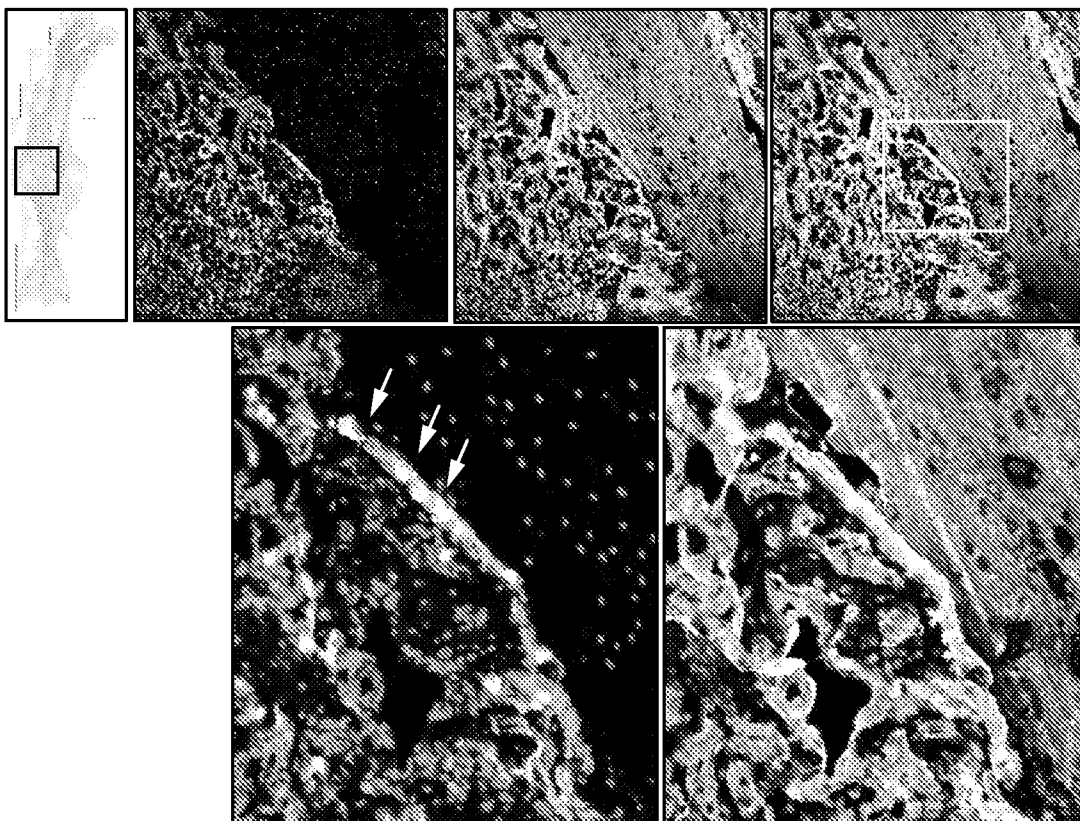
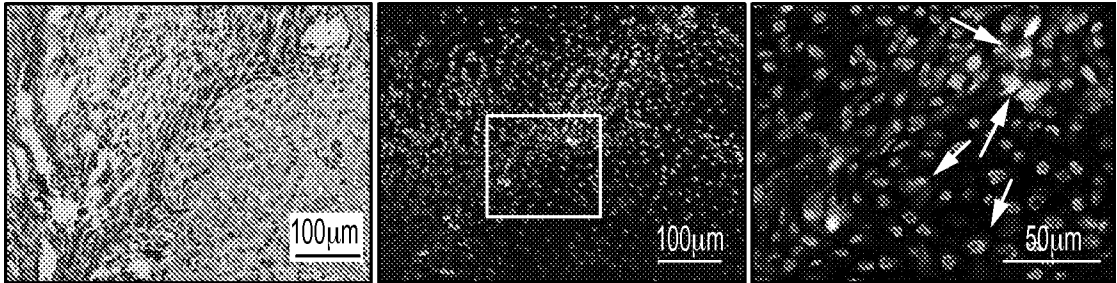


FIG. 12F

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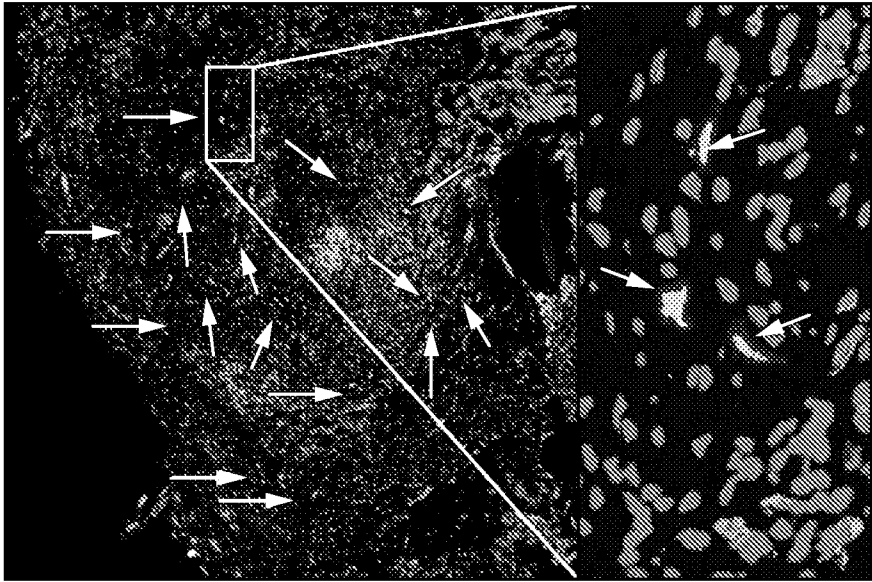
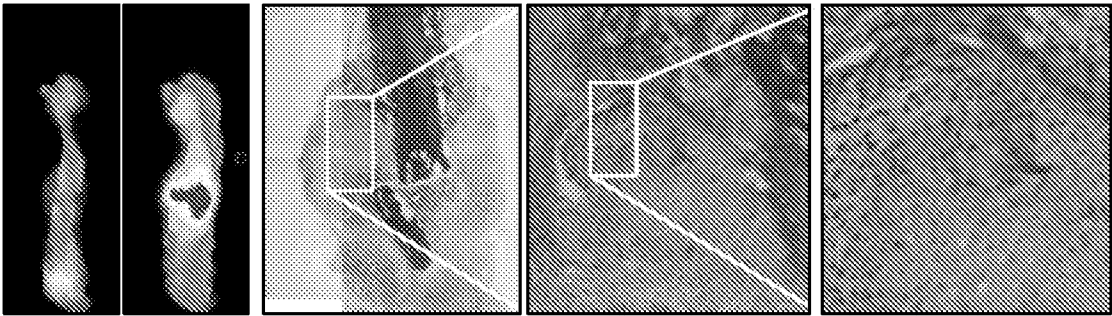


FIG. 13E

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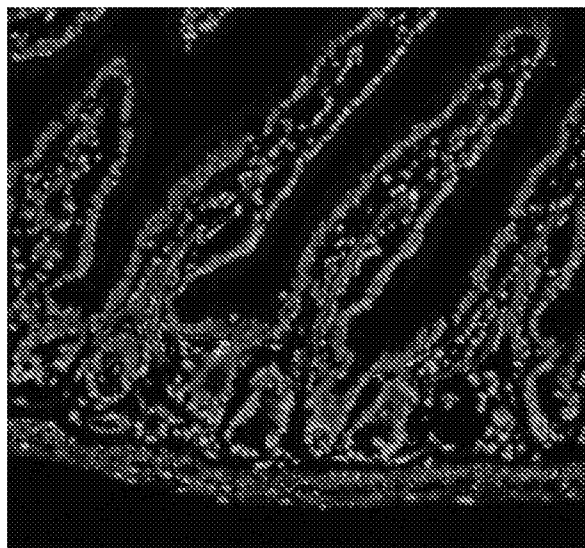


FIG. 14A

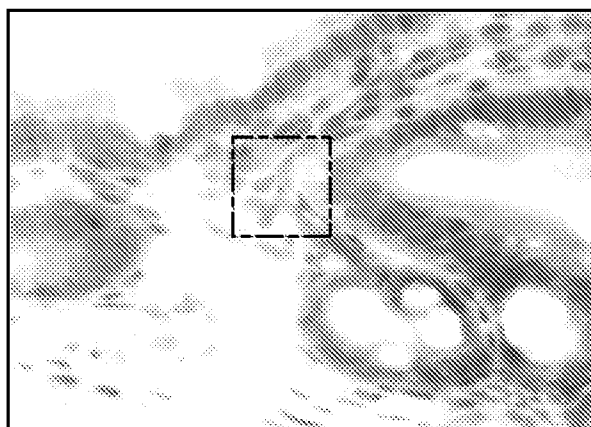


FIG. 14B

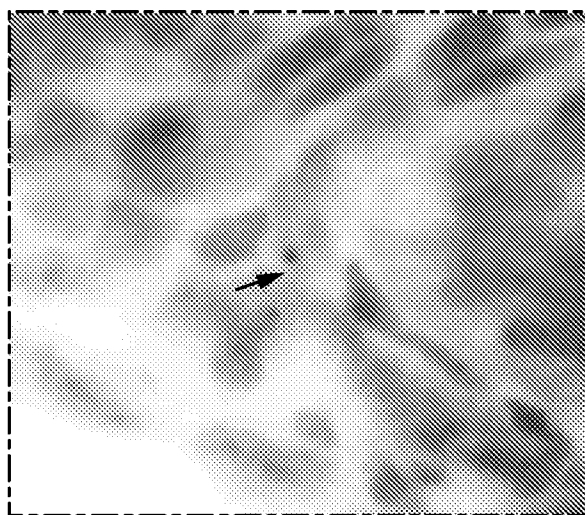


FIG. 14C

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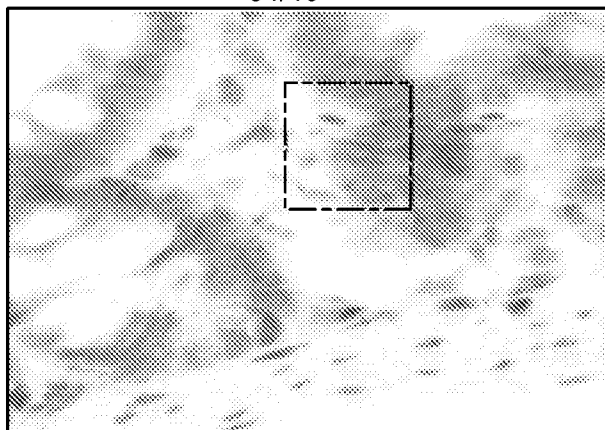


FIG. 14D

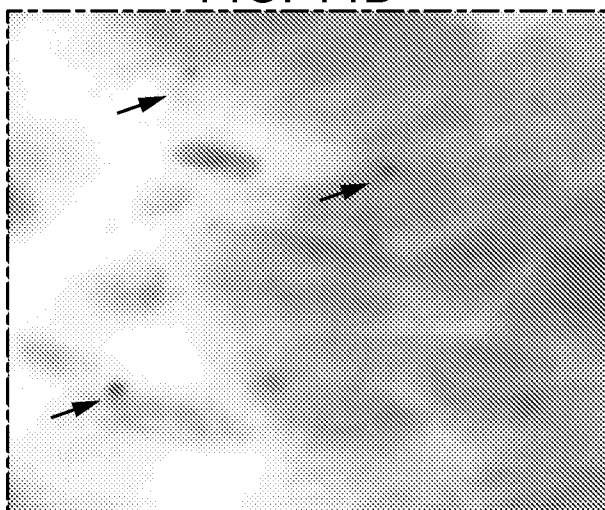


FIG. 14E

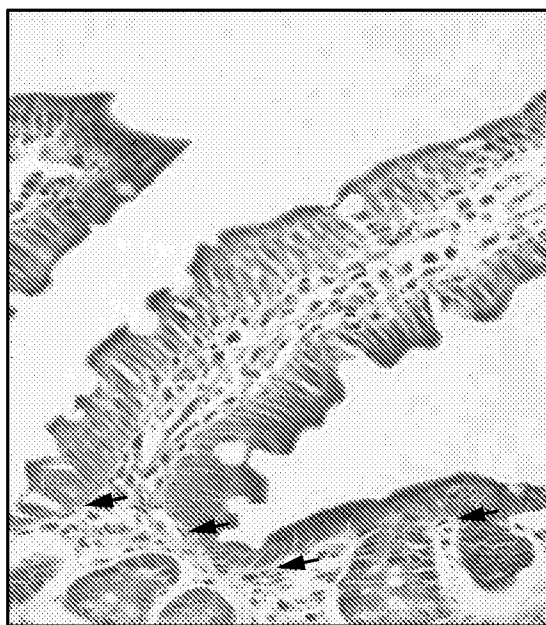


FIG. 14F

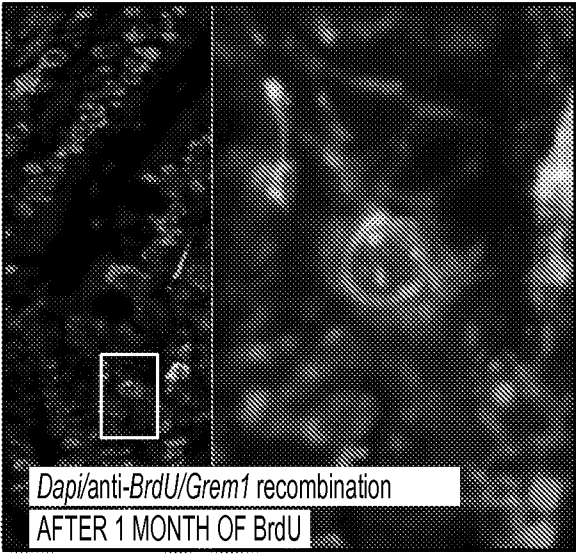


FIG. 14G

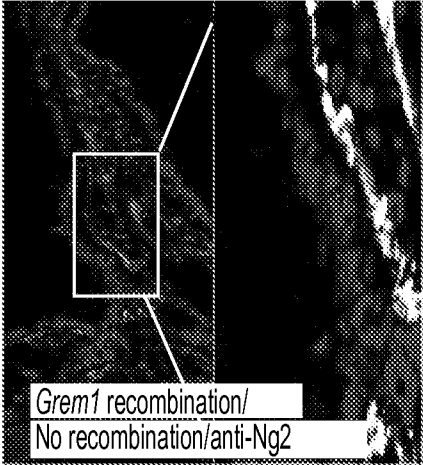


FIG. 14H

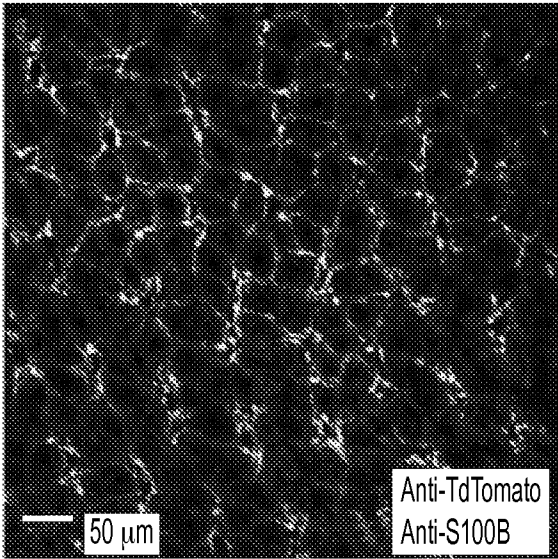


FIG. 14I

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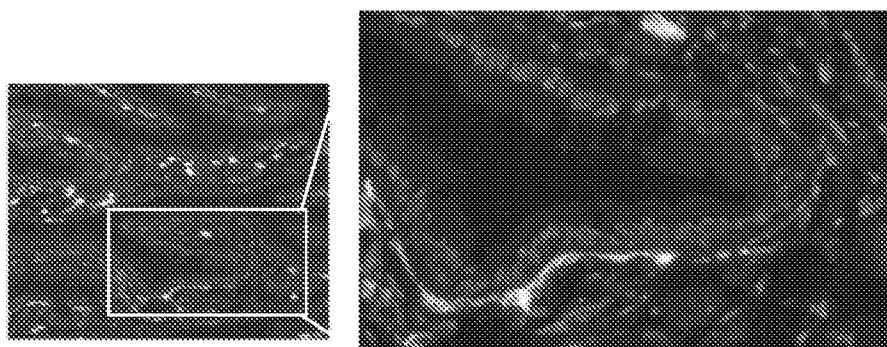


FIG. 14J

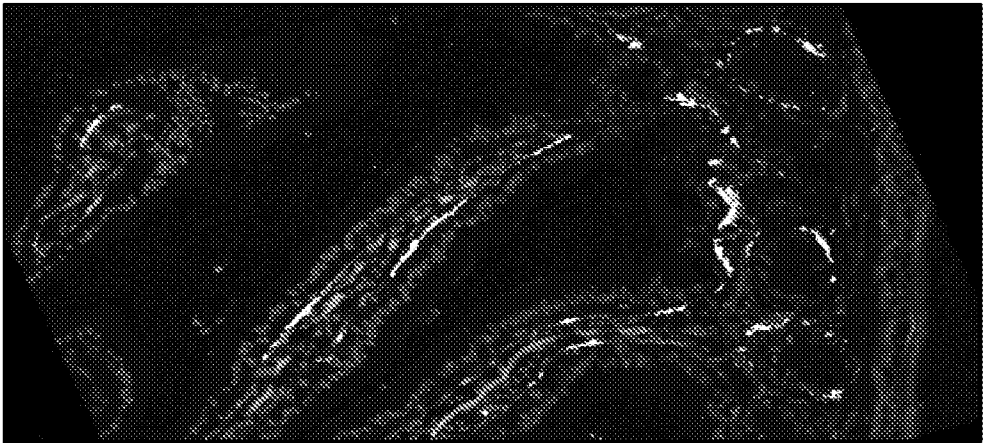


FIG. 14L

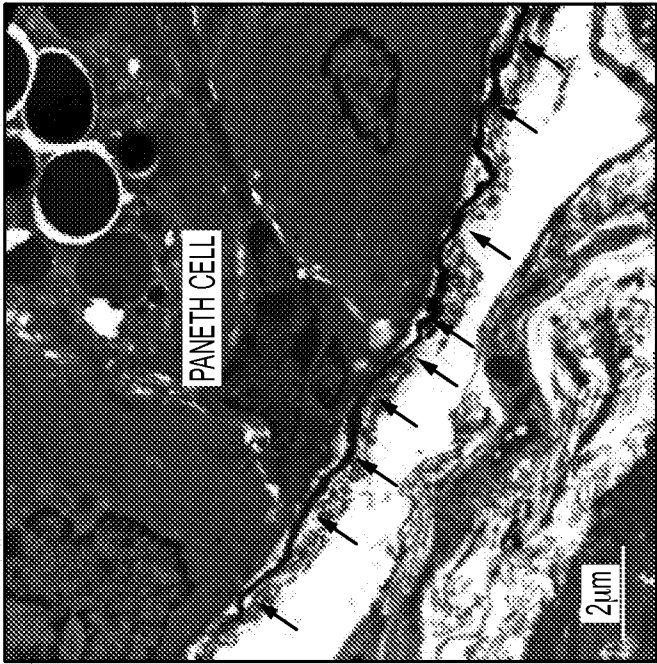


FIG. 14K

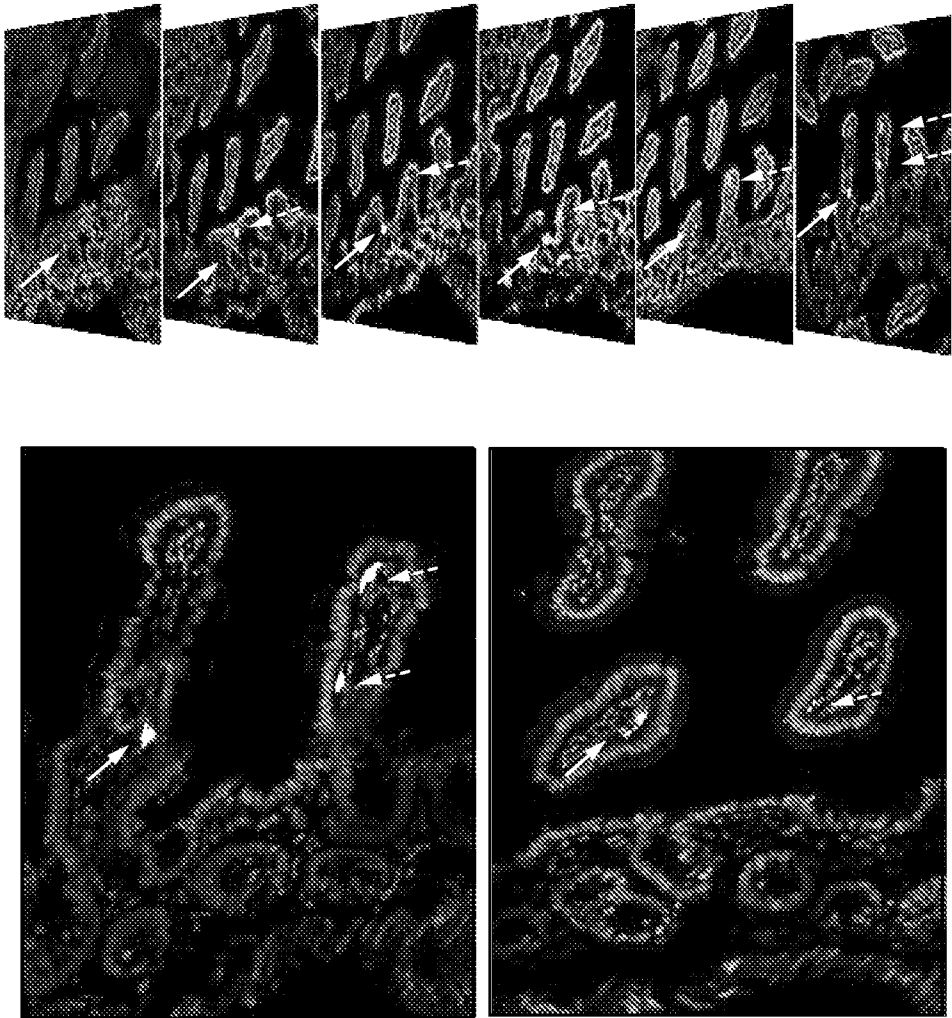


FIG. 14M

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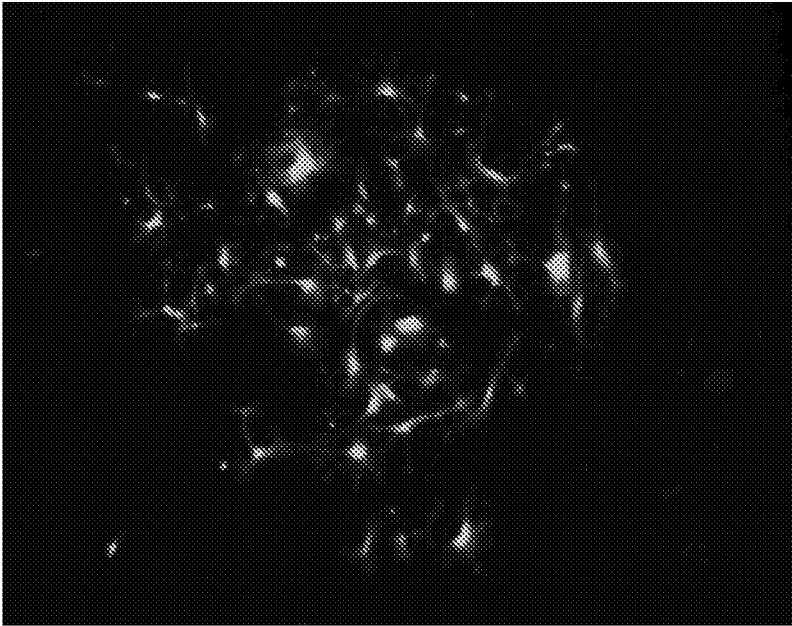


FIG. 14N

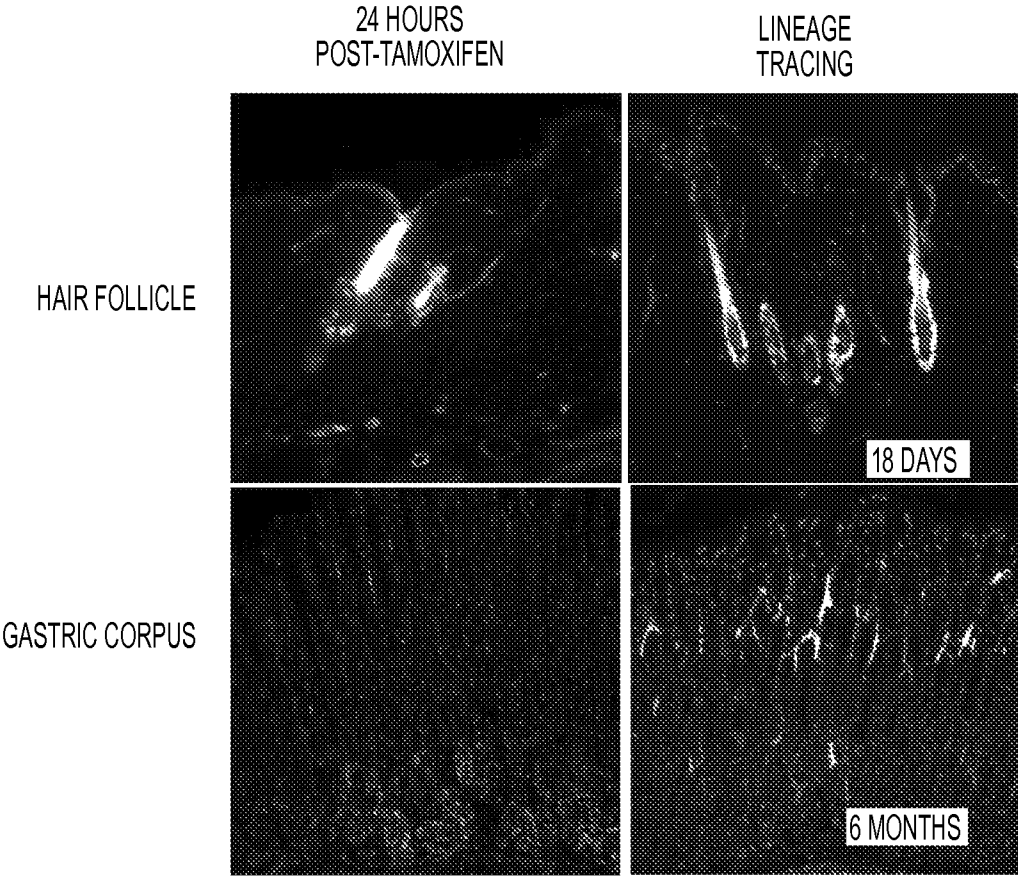


FIG. 14O

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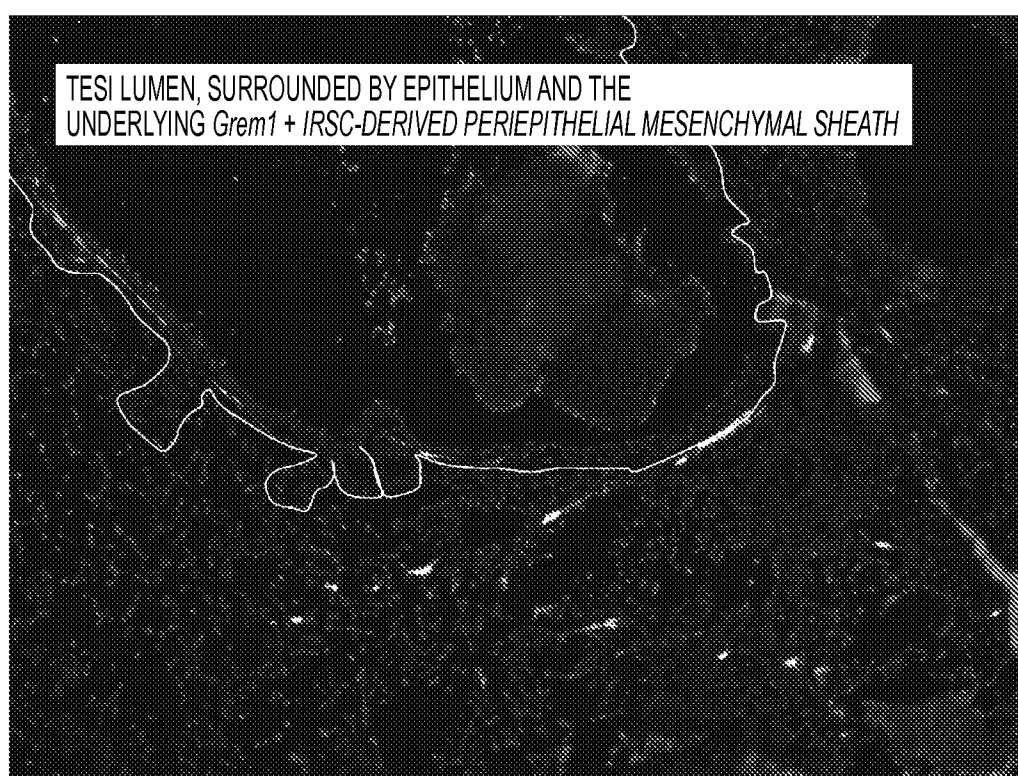


FIG. 14P