

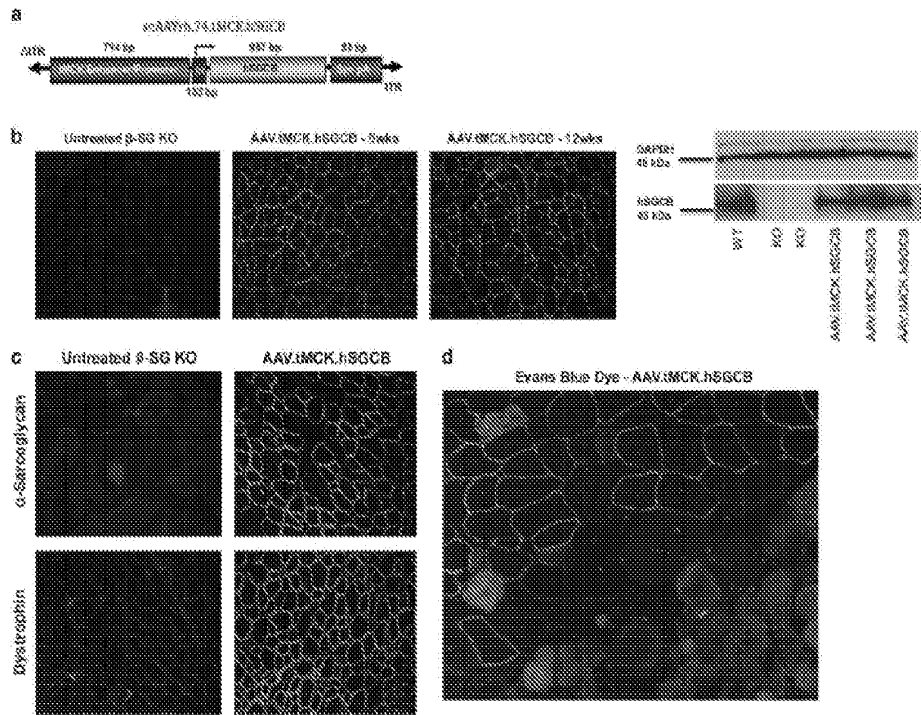


Figure 1. AAV mediated β -sarcoglycan expression restores dystrophin-associated proteins and protects membrane integrity. (a) Self-complementary AAV vector containing the codon-optimized human β -sarcoglycan gene (hSGCB) driven by the muscle-specific MCK promoter. The cassette also contains a chimeric intron to augment processing and polyadenylation signal for stability. (b) Immunofluorescence staining with anti- β -SG antibody shows high levels of sarcolemmal staining of the SGCB transgene in 3-week-old mice both 6 and 12 weeks post injection. $\times 20$ images shown. Percentage of fibers expressing beta-sarcoglycan per TA muscle averaged $28.4 \pm 4.2\%$ after 6 weeks ($n=9$, 4 male, 5 female) and $76.5 \pm 5.2\%$ after 12 weeks ($n=6$, 4 male, 2 female). Protein expression confirmed in the Western blot with gamma-tubulin blot shown for a loading control. (c) AAV delivery of β -sarcoglycan leads to restoration of other members of the sarcoglycan complex; α -sarcoglycan, dystrophin. $\times 20$ images. (d) scAAVrh.74.hSGCB protects $sgub^{-/-}$ membranes from damage. Image showing a large area of Evans blue-positive fibers (red) juxtaposed to a cluster of β -sarcoglycan-positive fibers that have been protected from Evans blue dye incorporation. $\times 40$ image is shown.

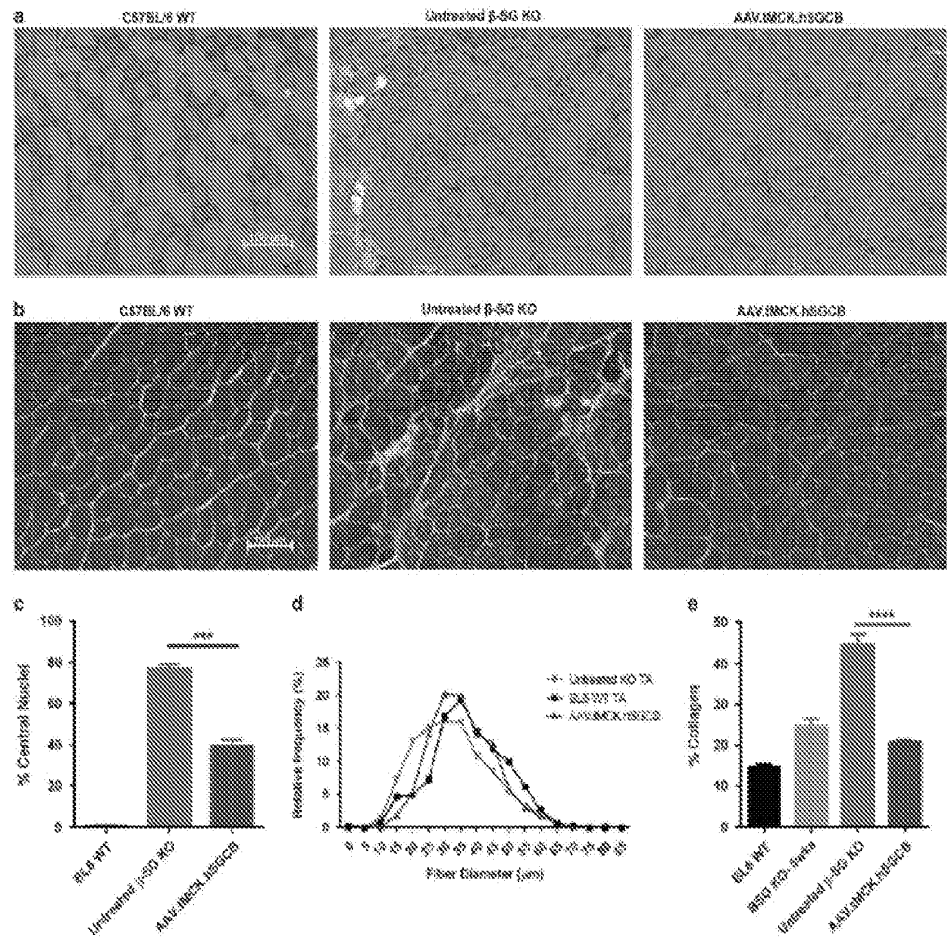


Figure 2. Histological analysis of β -5G-deficient treated skeletal muscle. scAAVrh.74.hSGCB treatment normalizes histological parameters of β -5G-deficient mice. Hematoxylin & Eosin staining and Picrosirius Red staining was performed on TA muscle from β -5G-deficient mice along with normal control C57/BL6 mice and scAAVrh.74.hSGCB-treated mice followed by quantification of histological parameters and % collagen staining. (a) H&E staining shows the presence of centrally nucleated fibers, inflammatory cells and large fiber diameter distribution in β -5G-deficient muscle and an improvement in histopathology following gene transfer. (b) Picrosirius Red staining shows a decrease in red collagen staining in treated muscle. (c) Quantification of centrally nucleated fibers showing a decrease following treatment ($P < 0.0005$, one-way ANOVA) and (d) representation of fiber size distribution and increase in average fiber size of TA muscle from C57/BL6 controls and β -5G-deficient mice compared with treated mice ($P < 0.0001$, one-way ANOVA). (e) Quantification of % collagen in TA muscle from C57/BL6 controls and β -5G-deficient mice compared with β -5G-deficient treated mice ($P < 0.0001$, one-way ANOVA). 100 μ m scale bar shown for $\times 20$ images. **** $P < 0.0001$.

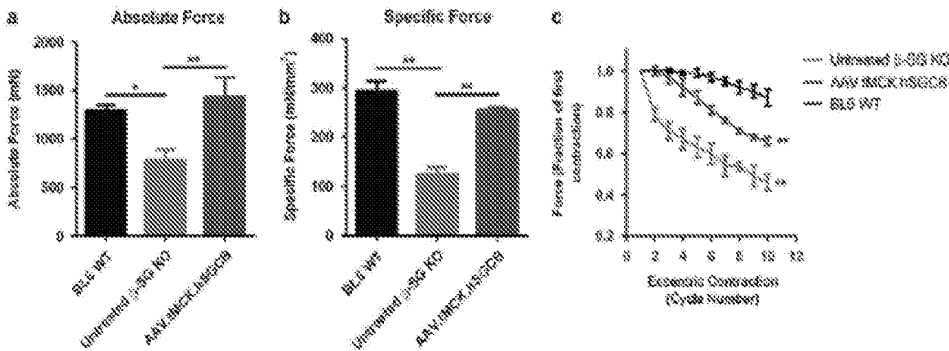


Figure 3. scAAVrh.74.hSGCB intramuscular delivery corrects tetanic force and resistance to contraction-induced injury. The TA muscle of β -sgb^{-/-} mice treated with 3×10^{12} vg of scAAVrh.74.hSGCB via an IM injection was harvested 6 weeks post gene transfer, and subjected to a protocol to assess tetanic force and an eccentric contraction protocol to assess resistance to contraction-induced injury. (a) AAVrh.74.hSGCB-treated TA's demonstrated significant improvement in both absolute tetanic force ($P < 0.01$, paired *t*-test) and (b) normalized specific force ($P < 0.05$, paired *t*-test), which was not different from wild-type force (C57/BL6). (c) AAVrh.74.hSGCB treated TA's exhibited significant improvement in resistance to contraction-induced injury compared with untreated β -sgb^{-/-} controls ($P < 0.01$, two-way ANOVA). Force retention following 10 contractions is shown. * $P < 0.05$; ** $P < 0.01$.

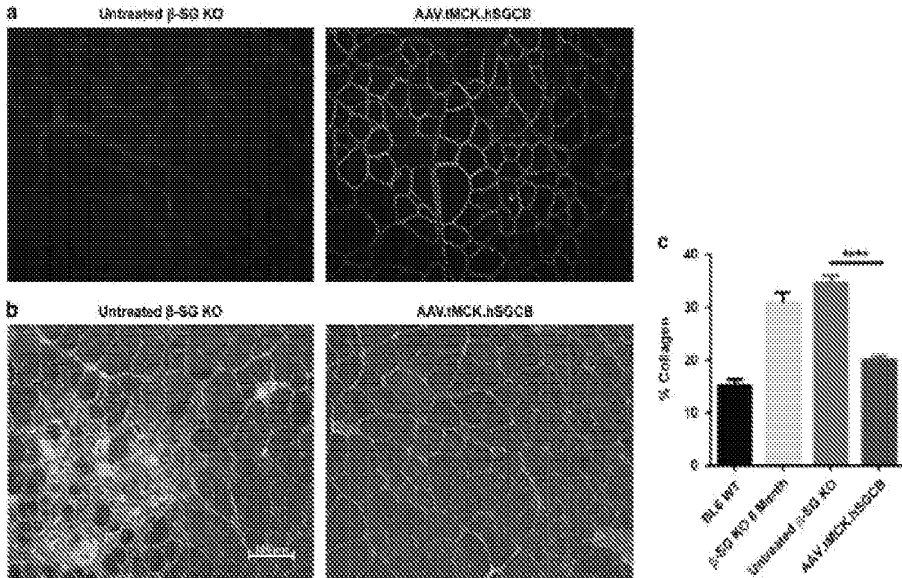


Figure 4. Analysis of aged mice treated intramuscularly with scAAVrh.74.hMCK.hSGCB. (a) Immunofluorescence staining of TA muscle from 6-month-old treated β -sgb^{-/-} mice 12 weeks post injection ($n = 5$, 5 male) shows sarcolemmal expression of the SGCB transgene at levels averaging 80% in injected mice compared with untreated ($n = 4$, 4 male). (b) Picrosirius red staining of the treated and untreated TA muscle. (c) Quantification of collagen present in the Picrosirius red stained tissue shows a significant reduction in the amount of collagen following treatment with scAAVrh.74.hMCK.hSGCB ($P < 0.0001$, one-way ANOVA). 100 μ m scale bar shown for $\times 20$ images. **** $P < 0.0001$.

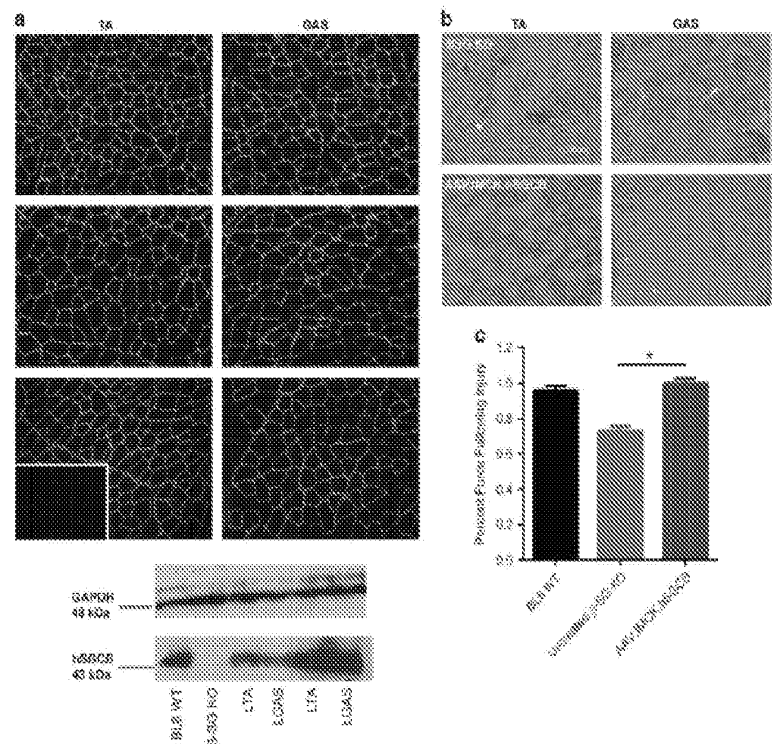


Figure 5. Vascular delivery of scAAVrh.74.hSGCB. Four ($n = 5$, 3 male) and five ($n = 4$, 2 male, 2 female) weeks β -5G-deficient mice were treated with vector via the femoral artery to deliver the vector to the lower limb muscles. At a dose of 5×10^{11} vg, β -5G expression was $80.6 \pm 3.8\%$ in the TA and $91.8 \pm 4.7\%$ in the GAS of treated mice accompanied by improvements in histopathology that resulted in significant improvement in specific force compared with untreated animals even following an injury paradigm. (a) β -5G protein expression from three representative mice. Muscle from a β -5G KO untreated mouse is shown for comparison in the inset (lower right). $\times 20$ Images are shown. Expression in treated muscles confirmed via western blot and gamma-tubulin is shown as a loading control. (b) Histopathology is significantly improved following high dose treatment. Upper panels-treated TA and gastrocnemius muscles. Bottom panels-untreated β -5G-deficient control muscle. 100 μ m scale bar shown for $\times 20$ images. (c) Percentage of specific force retained in EDL muscle following 10 cycles of eccentric contraction-induced injury. Treatment with 5×10^{11} vg of AAVrh.74.hSGCB led to significant improvement in force that was equivalent to WT (normal) control muscle ($P < 0.05$, one-way ANOVA). * $P < 0.01$.

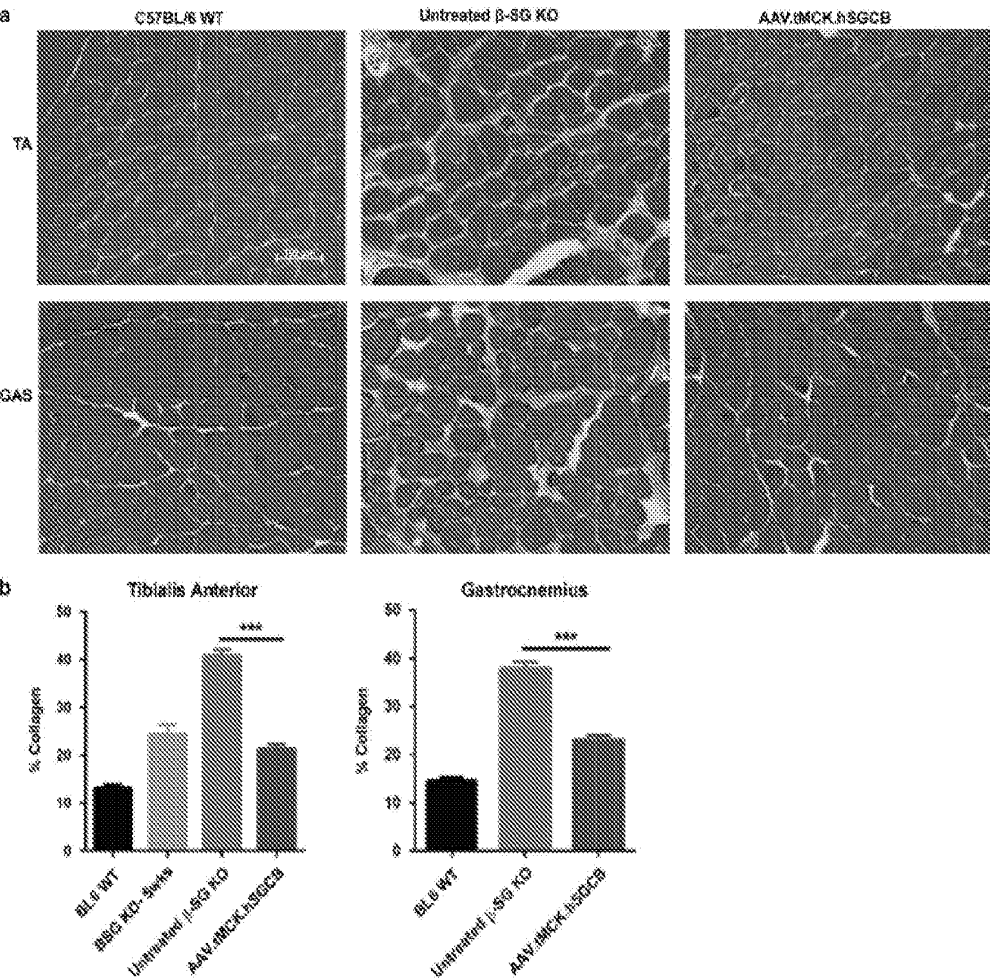


Figure 6. Reduction of fibrosis in ILP-treated β -SG KO mice. (a) Picrosirius red staining shows reduced fibrosis in treated mice indicated by a decrease in collagen deposition compared with untreated β sgb^{-/-} mice. (b) Quantification of collagen levels in the TA and GAS muscles from BL6 WT, untreated β sgb^{-/-} mice, and treated mice confirm reduction in collagen levels in treated mice ($P < 0.001$, one-way ANOVA). 100 μ m scale bar shown for $\times 20$ images. *** $P < 0.001$.

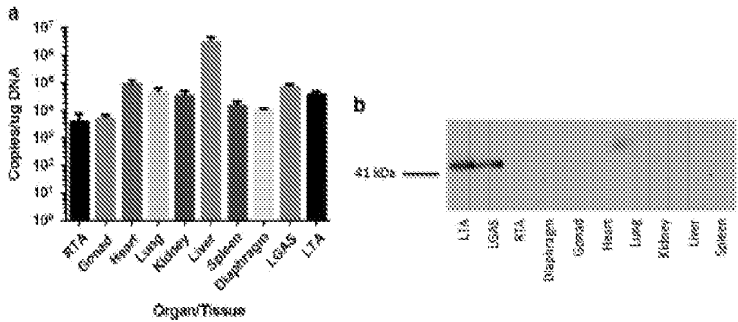


Figure 7. Vector biodistribution and protein expression. (a) Histogram of average distribution of vector in harvested tissues from ILP-treated mice given in copies of transcript per microgram of DNA added to qPCR. Left limb was treated. (b) No protein expression via western blot seen in off target organs.

Figure 8A



Figure 8C

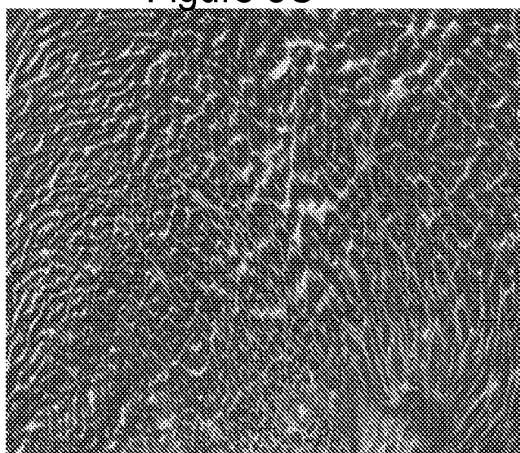


Figure 8B

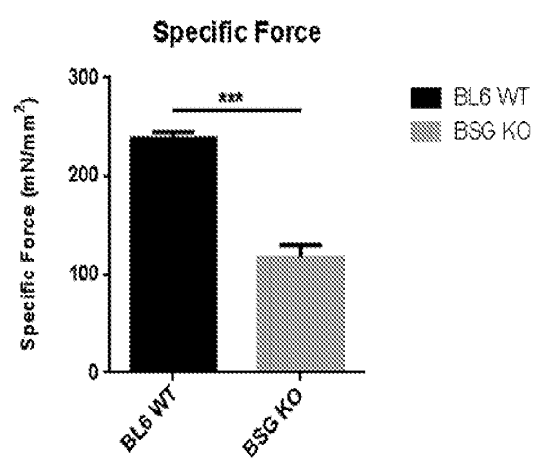


Figure 8D

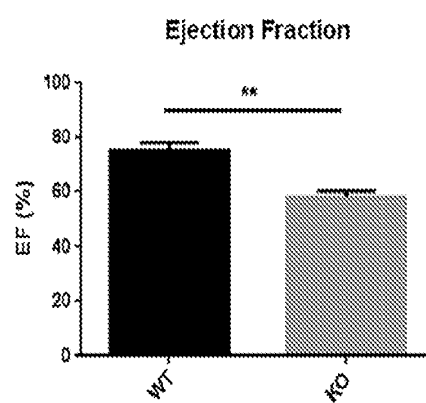


Figure 9

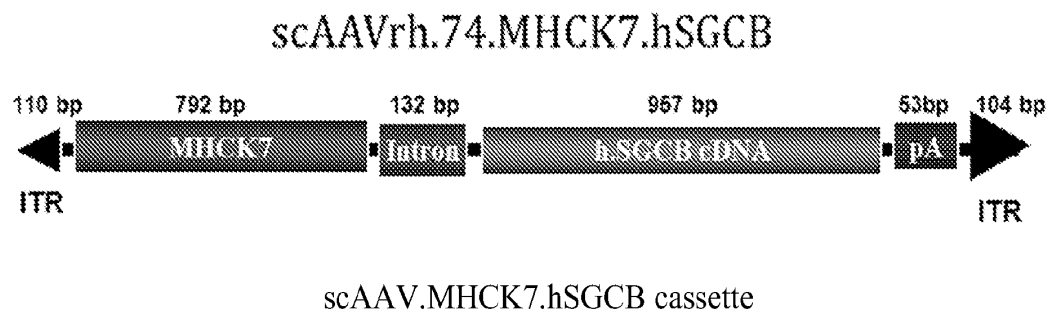


Figure 10

Delivery of scAAVrh.74.MHCK7.hSGCB via the tail vein of SGCB-/- mice results in nearly 100% transduction of muscle fibers in every muscle

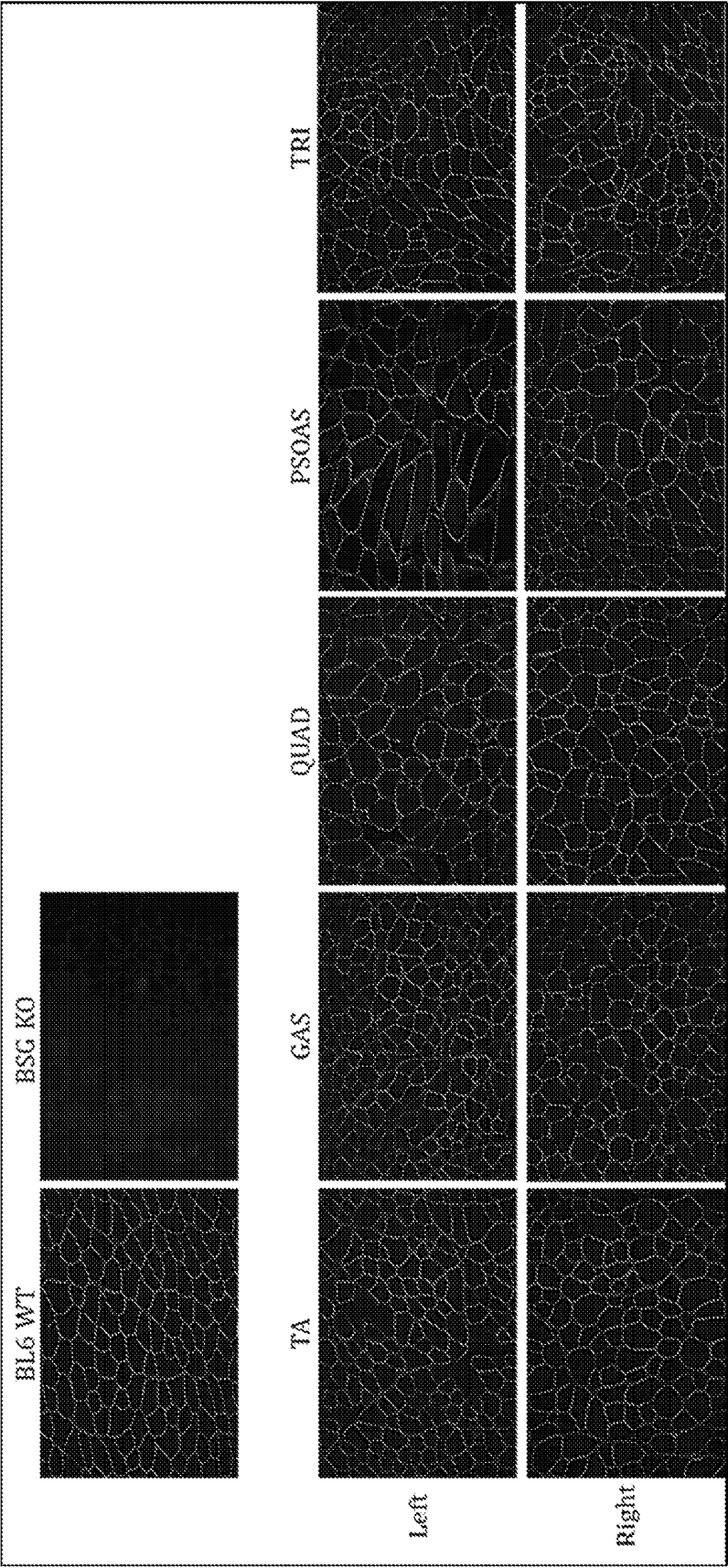


Figure 11

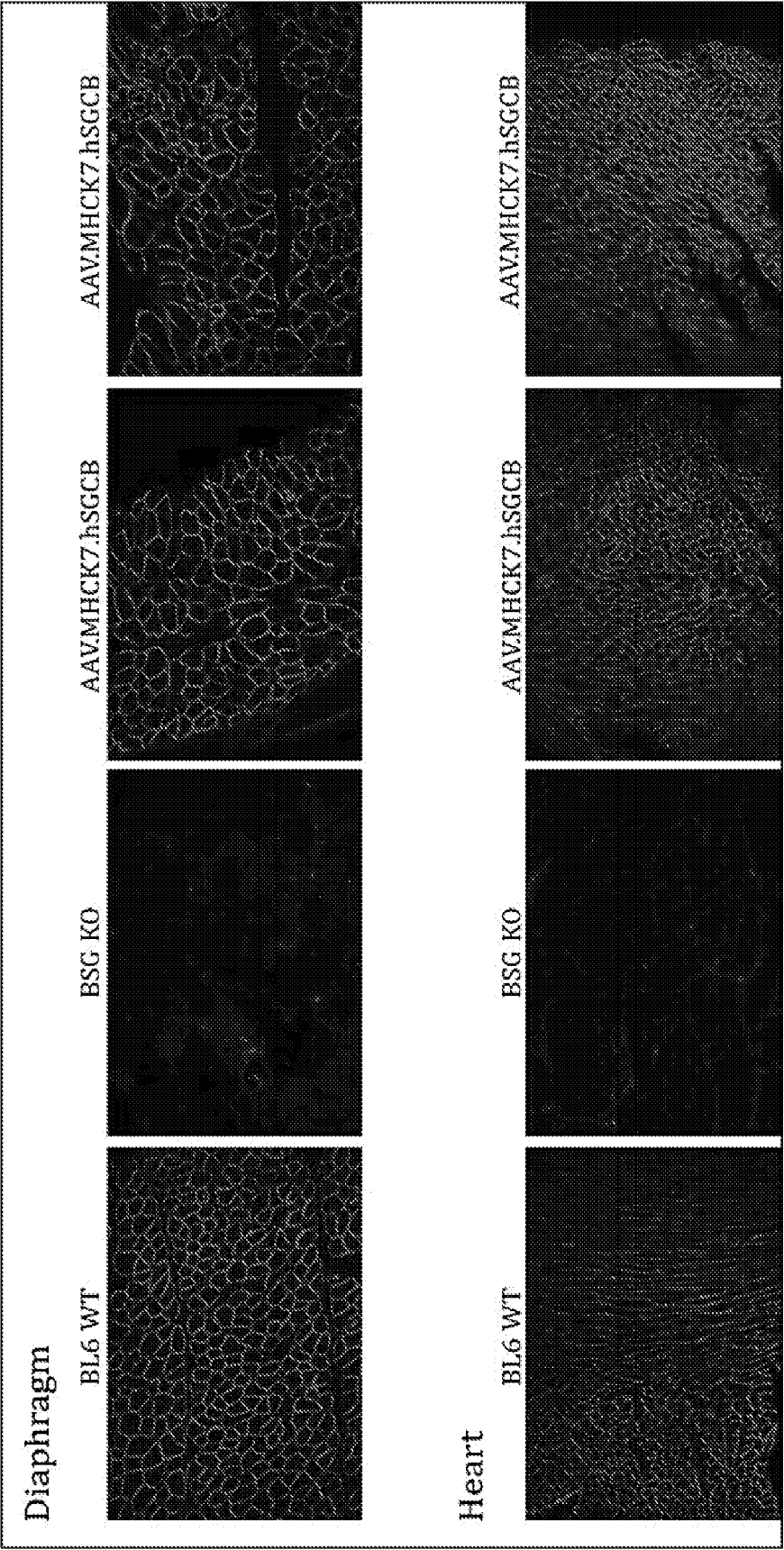


Figure 12B

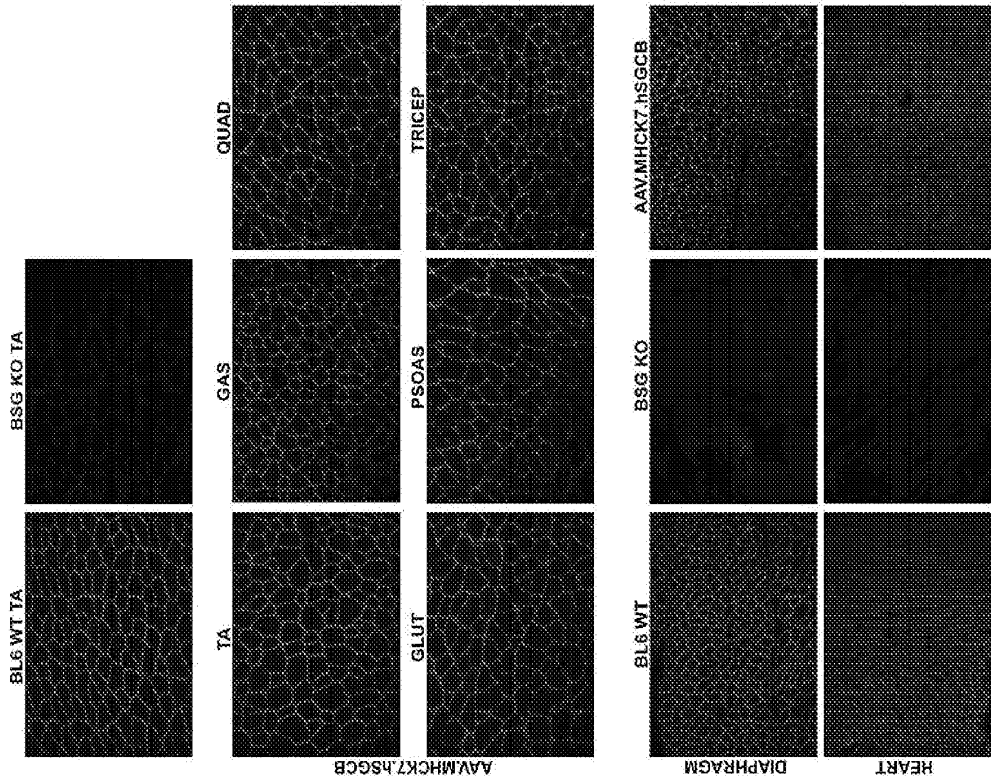


Figure 12A

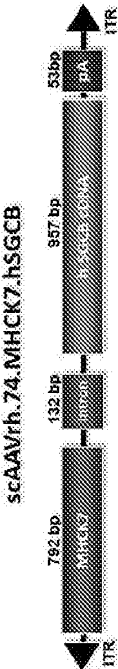


Figure 12C

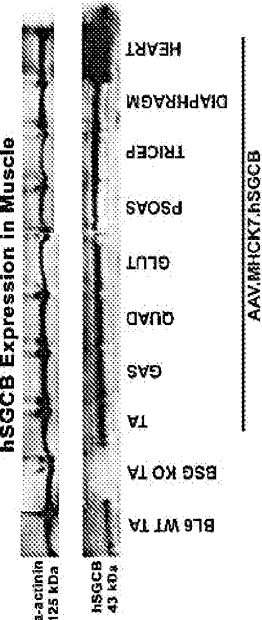


Figure 12D

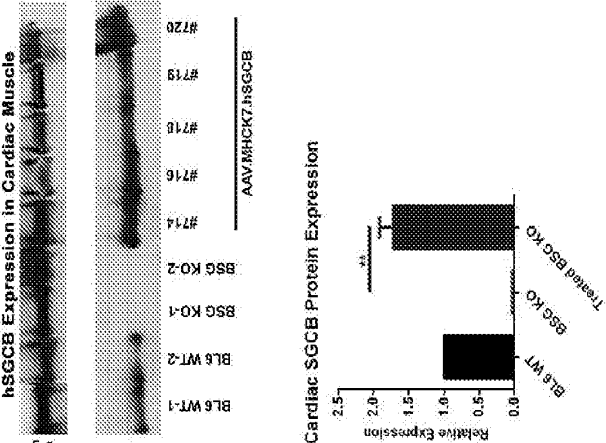


Figure 13A

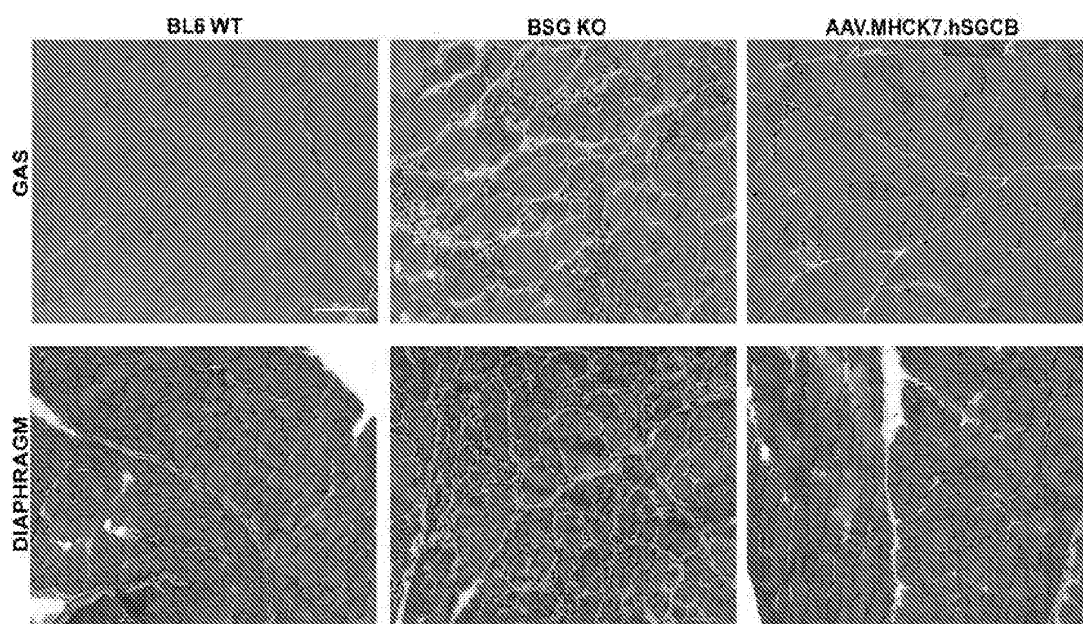
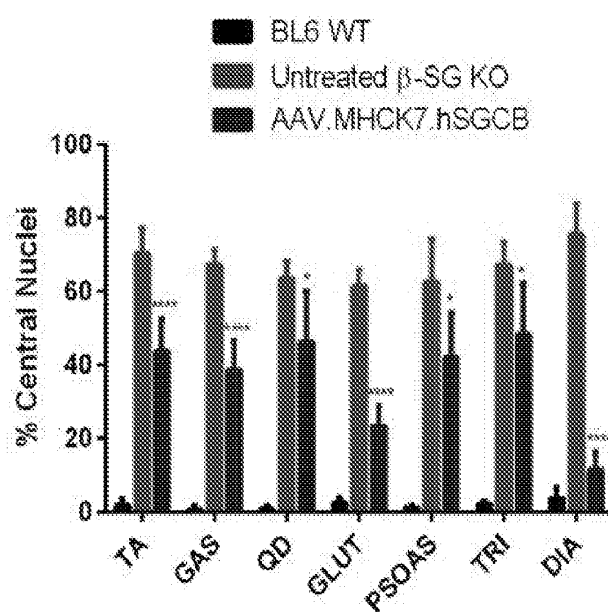


Figure 13B



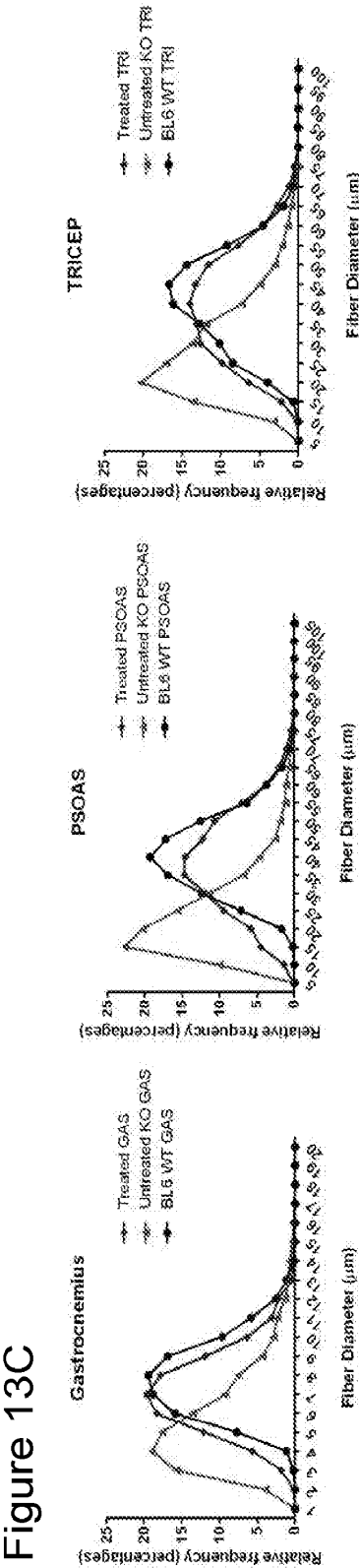
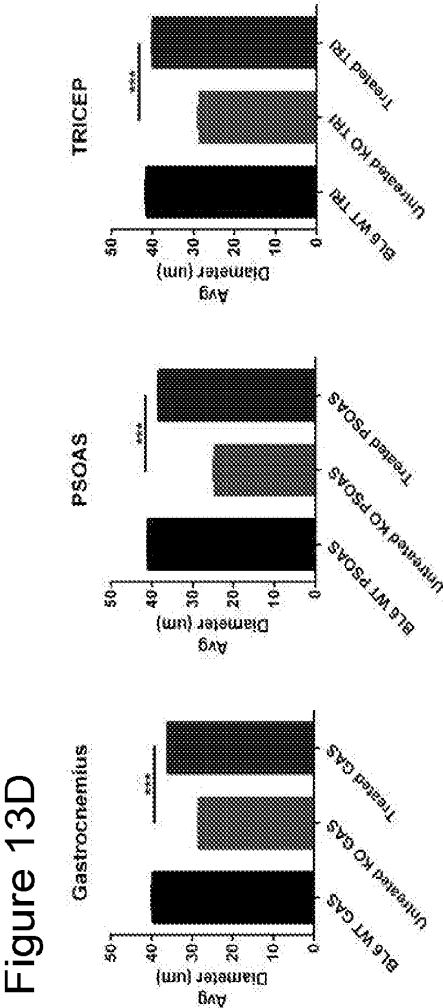


Figure 14A

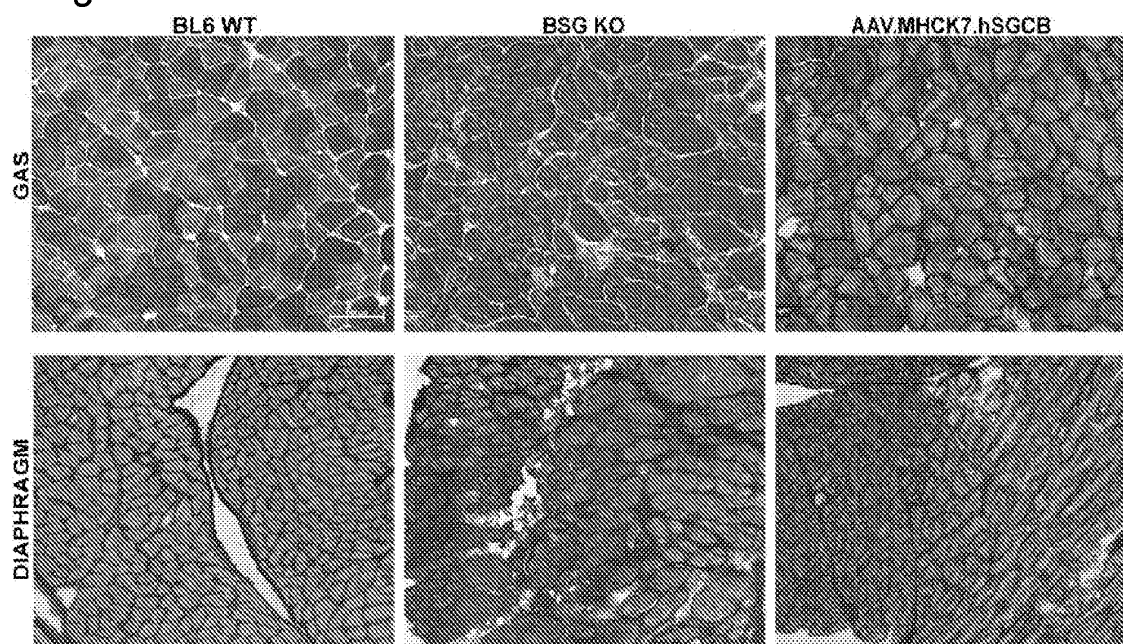


Figure 14B

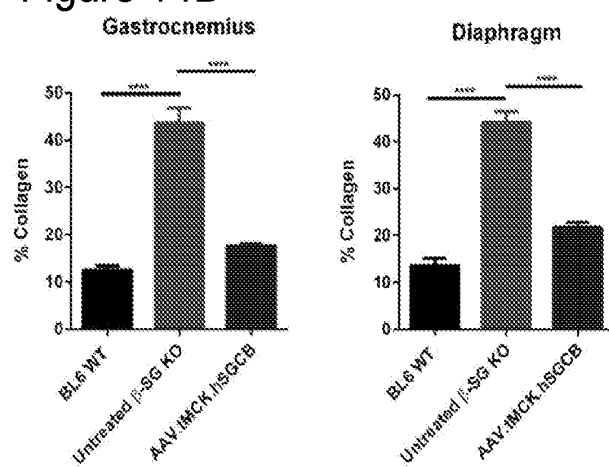


Figure 15
MHCK7.hSGCB 6 month IV 1e12vg

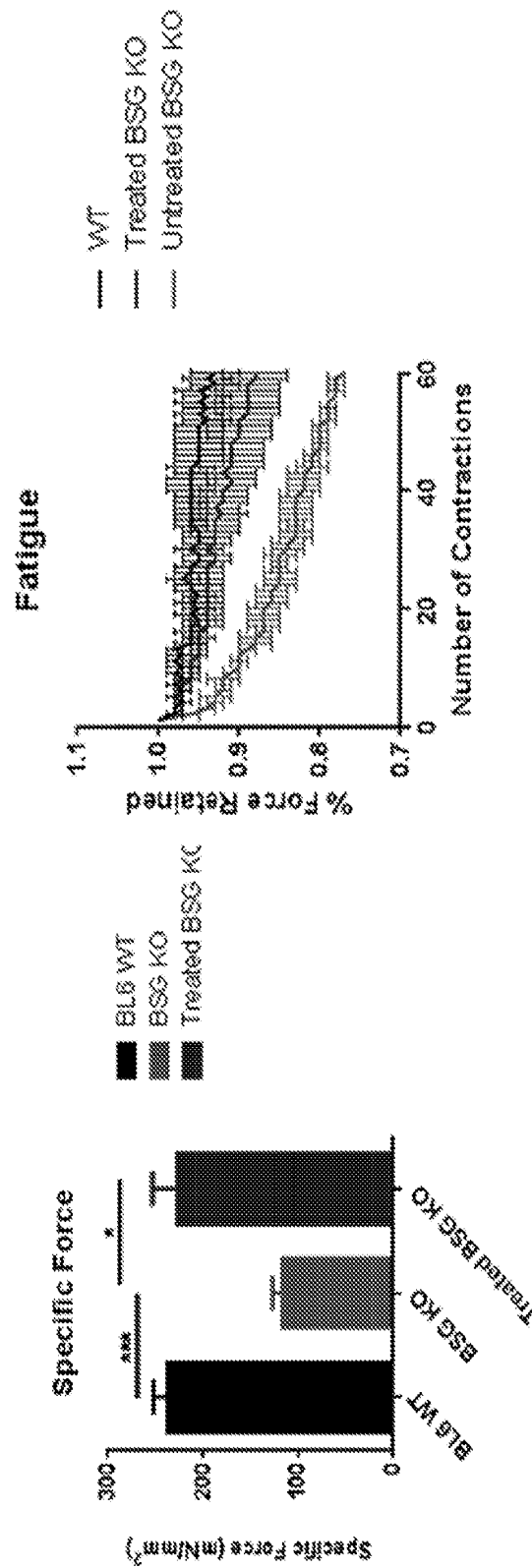


Figure 16



scAAVrh.74.CMV.miR29c

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Figure 17

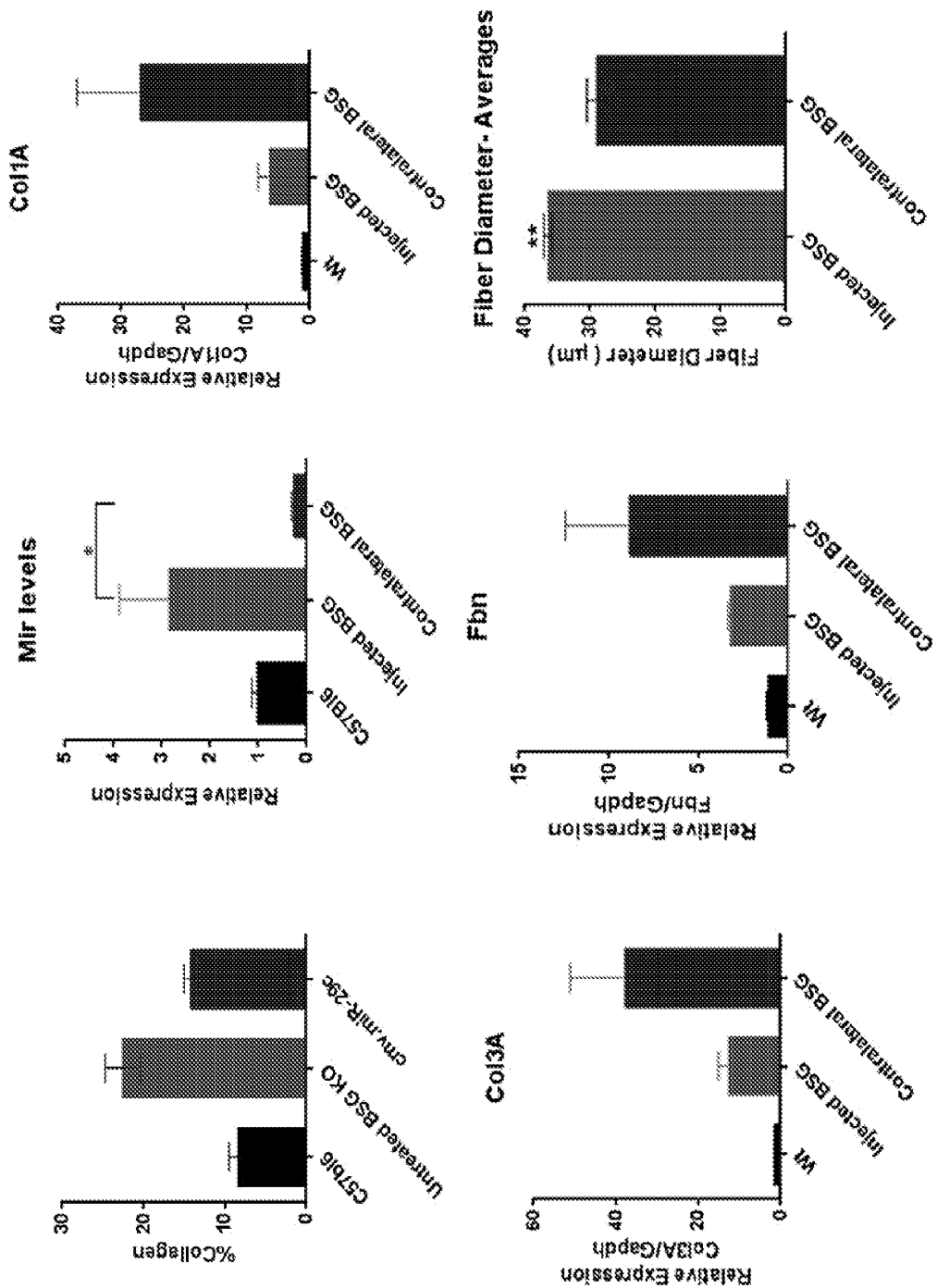
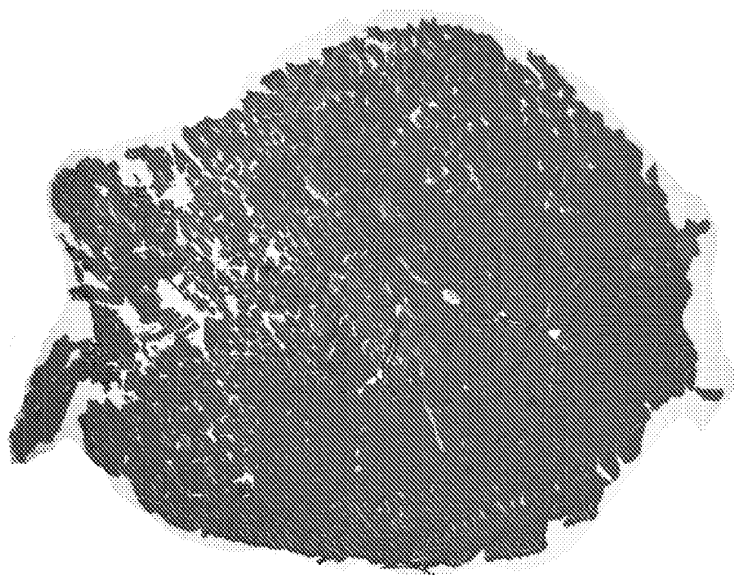
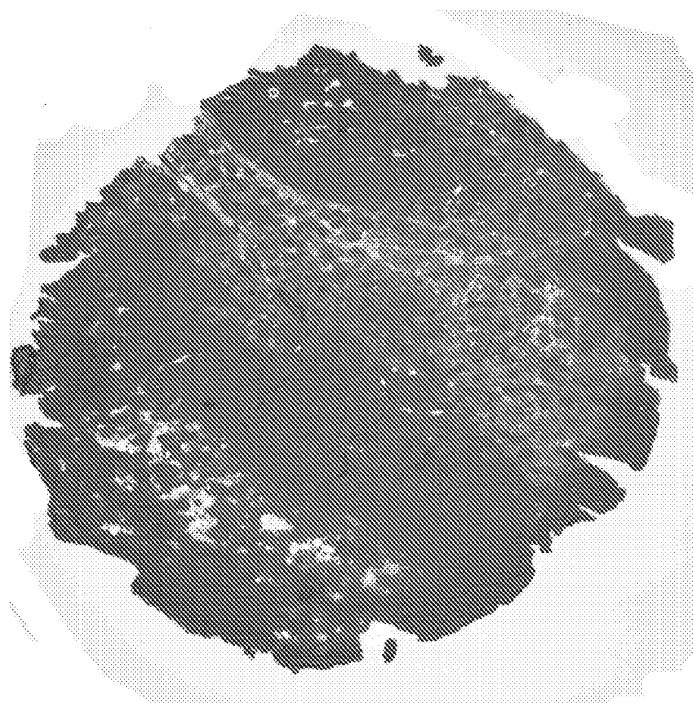


Figure 18

AAVrh.74.CMV.mir29C treated SGCB-/-



Untreated SGCB-/-



INCORPORATED BY REFERENCE (RULE 20.6)
SUBSTITUTE SHEET (RULE 26)

Figure 19A

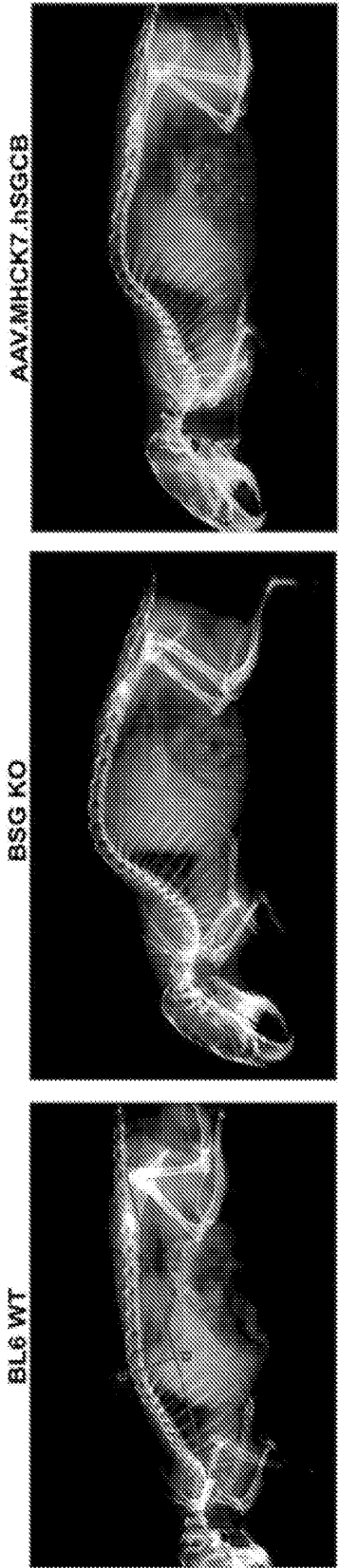


Figure 19B

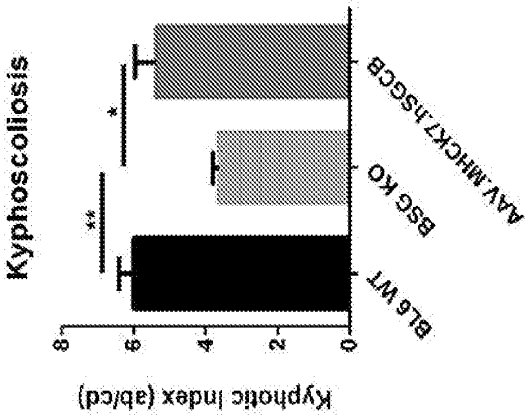


Figure 20A

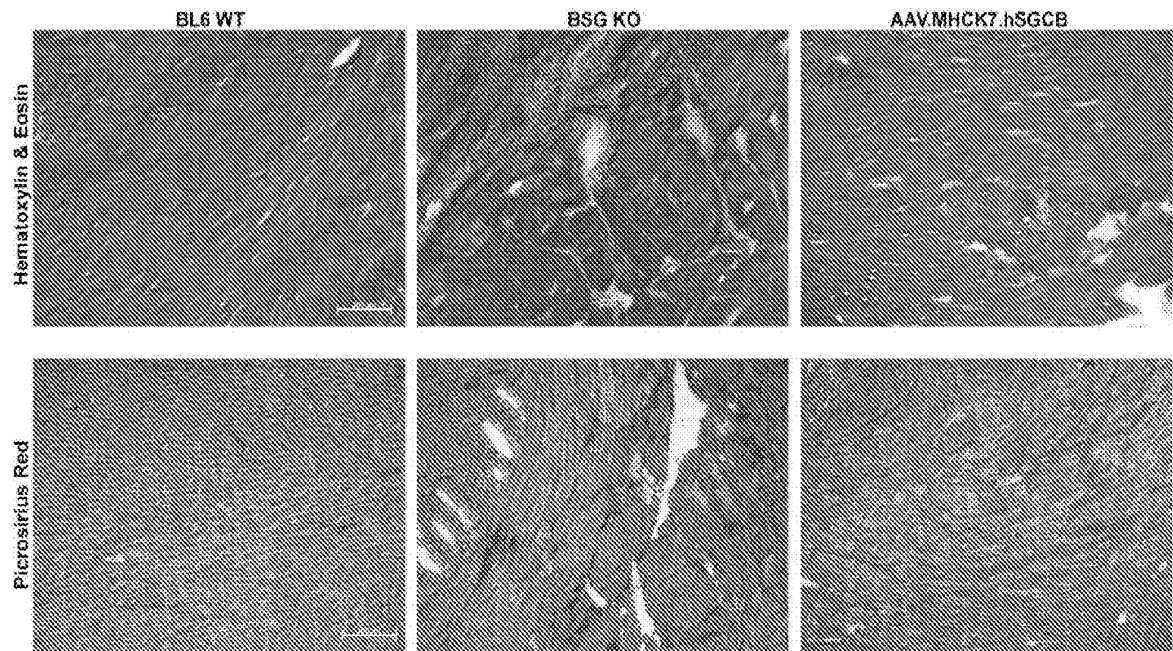


Figure 20B

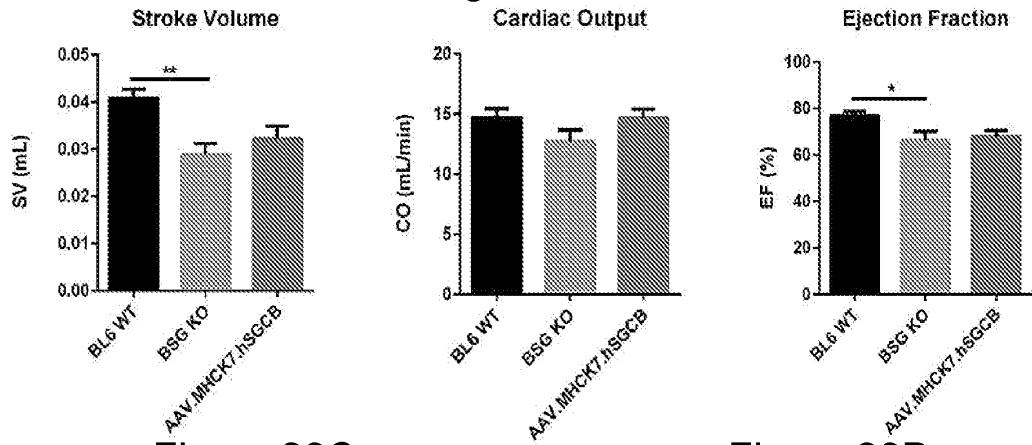


Figure 20C

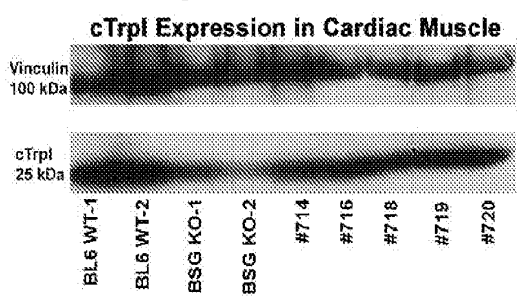


Figure 20D

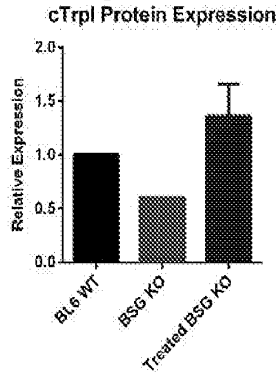


Figure 21A

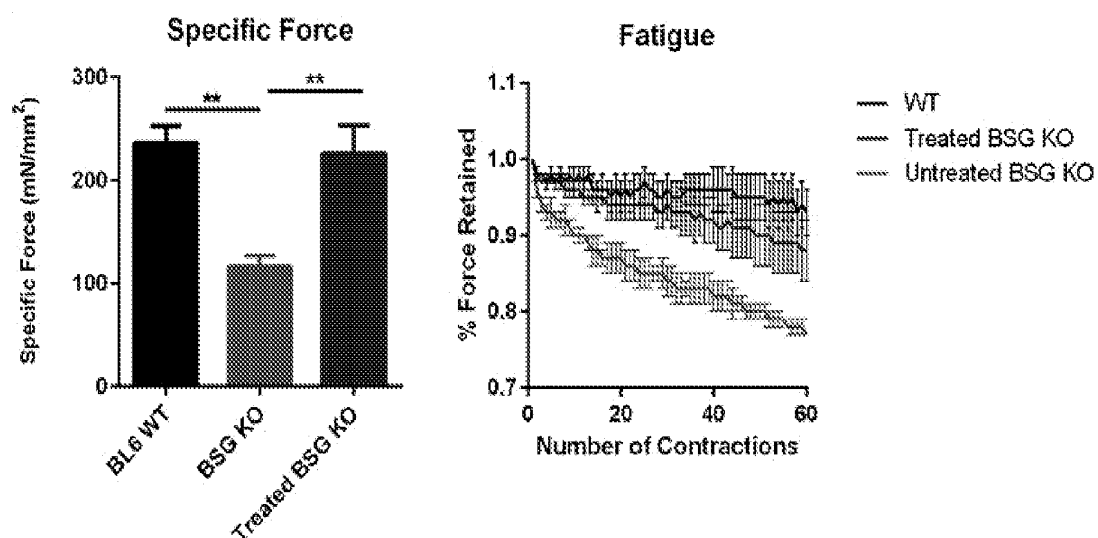


Figure 21B

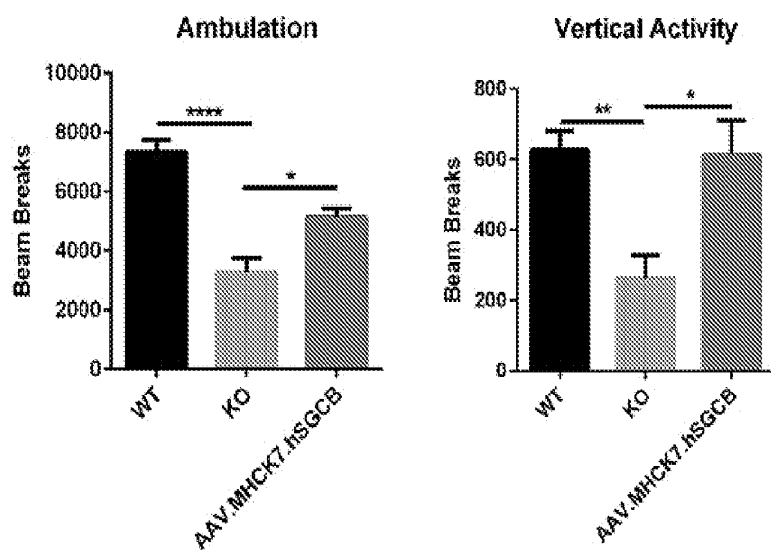


Figure 22B

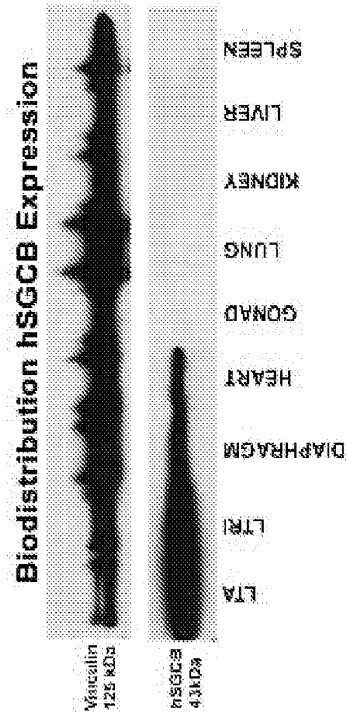


Figure 22A

