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Supplemental Information

Neonatal Systemic AAV-Mediated Gene Delivery

of GDF11 Inhibits Skeletal Muscle Growth

Quan Jin, Chunping Qiao, Jianbin Li, Juan Li, and Xiao Xiao

hGDF11

5**′ -**

atggtgctggctgcccccctgctgctgggatttctgctgctggctctggaactgcggcctagaggcgaagccgctgacccctagcqtqqcccctqaqcctqatqqtcctqtqtqcqtqtqqcqqcaqcactccaqaqaqctqcqqctqqaa tccatcaagagccagatcctgagcaagctgagactgaaagaggcccccaacatcagccgcgaggtcgtgaaacagct gctgcccaaagccccccactgcagcagattctggacctgcacgacttccaggggggacgccctgcagcccgaggatt tcctqqaaqaqqacqaqtaccacqccaccaccqaqacaqtqatcaqcatqqcccaqqaaaccqaccccqccqtqcaq acagatggcagccctctgtgctgccacttccacttcagccccaaagtgatgttcacccaaggtgctgaaagcccagct qtqqqtqtacctqaqqcccqtqcctaqaccaqccaccqtqtacctqcaqatcctqcqqctqaaqcctctqaccqqcq aqqqaacaqctqqcqqcqqaqqqaqqaaqaaqqcacatcaqaatcaqaaqcctqaaqatcqaqctqcacaqcaqa agcggccactggcagagcatcgacttcaaacaggtgctgcactcctggttccggcagccccagagcaattggggcatcgagatcaacgccttcgaccccagcggcaccgatctggccgtgacatctctggggacctggcgccgaaggcctgcaccccttcatqqaactqaqaqtqctqqaaaacaccaaqcqqaqccqqcqqaacctqqqcctqqattqtqatqaqcacaqc agcgagagccggtgctgcagataccccctgaccgtggacttcgaggcctttggctgggactggatcattgcccccaa gcqgtacaaqqccaactactqcaqcqqccaqtqcqaqtacatqttcatqcaqaaqtacccccacacccaqtqqtqc agcaggccaatcctagaggctctgccggcccttgctgcacccccacaagatgagccccatcaacatgctgtacttc aacgacaagcagcagatcatctacggcaagatcccccggcatggtggtggacagatgcggctgctcc-3'

hGDF11Pro-Fc-1

5**′ -**

gageteaagettaagetaacaaagaceaegaegatateaeggtegtggteteaaagaacaaeaaaaagteeg actgagaaggagccaccatggtgctggctgctcctctgctgctgggattcctgctgctggccctggaactgaggcctagaggcgaagccgctgaaggacctgctgccgctgccgccgccgctgcagcagcagctgcaggcgttggcggaga gagatetageagacetgececettetgtggececetgageetgatggetgteetgtgtgtgtgtgggggeageageagea gagagctgcggctggaatccatcaagagccagatcctgagcaagctgagactgaaagaggcccctaacatcagcaga gaggtggtcaagcagctgctgcccaaggcccctccactgcagcagatcctggacctgcacgatttccagggcgacgc tctgcagcccgaggacttcctggaagaggatgagtaccaccgccaccaccgagacagtgatcagcatggcccaagaga ${\tt cagaccccgccgtgcagacagatggcagccctctgtgctgccacttccacttcagcccccaaagtgatgttcaccaag}$ gtgctgaaggcccagctgtgggtgtacctgaggcctgtgcctagacccgccaccgtgtatctgcagatcctgcggct qaaqcctctqaccqqcqaqqqaacaqctqqtqqcqqtqqcqqaqqacqqcqqcacatcaqaatcaqaaqcctqaaqa $\verb+tcgagctgcactccagaagcggccactggcagagcatcgacttcaaacaggtgctgcacagctggttccggcagccc$ cagagcaactggggcatcgagatcaacgccttcgaccccagcggcaccgatctggccgttacatctctgggacctggcqccqaqqqcctqcatccctttatqqaactqcqqqtqctqqaaaacaccaaqqaccaaqacccacacctqtcctccct gccctgcccctgaactgctgggcggacctagcgtgttcctgttcccaacgcccaaggacaccctgatgatcagccggacccctgaagtgacctgcgtggtggtggacgtgtccccacgaggatcccgaagtgaagttcaattggtacgtggacqqcqtqqaaqtqcacaacqccaaqaccaaqaccaqqaqqaacaqtacaacaqcacctaccqqqtqqtqtccqtqc tgaccgtgctgcaccaggactggctgaacggcaaagagtacaagtgcaaaggtgtccaacaaggccctgcccca atcgagaaaaccatcagcaaggccaagggccagccccgcgagcctcaggtttacacactgcctcccagccgggaaga gatgaccaagaaccaggtgtccctgacatgcctggtcaagggcttctaccccagcgatatcgccgtggaatgggaga gcaacqqccaqcctqaqaacaactacaaqaccacccctcccqtqctqqacaqcqacqqctcattcttcctqtacaqc aagctqaccqtqqacaaqaqccqqtqqcaqcaqqqcaacqtqttcaqctqctctqtqatqcacqaqqccctqcacaa ccactacacccagaagtccctgtccctgagccccggcaagtgatgactcgaggcggccgc-3'

Supplemental Figure 1. Codon-optimized sequences for human GDF11 and human GDF11Pro-Fc-1.



Supplemental Figure 2. (A) Vector construction. **(B)** Western blot analysis of cell lysates after vector reinfection with AAV9-GDF11 or AAV9-GDF11Pro-Fc-1 in HEK293 cells at a MOI of 10,000 vg/cell. AAV9-EGFP and PBS were used as control. **(C)** Representative immunofluorescence analysis confirmed the presence of the GDF11 and GDF11Pro-Fc-1 proteins after vector reinfection in HEK293 cells at a MOI of 10,000 vg/cell. 5 to 10 μ M Hoechst 33342 was co-administered into HEK293 cells to facilitate transgene expression.



Supplemental Figure 3. Representative immunofluorescence sections from PBS-treated or AAV9-GDF11-treated diaphragm stained for laminin- α 2 to visualize myofibers. Scale bar = 100 μ m.



Supplemental Figure 4. Total and normalized mass of abdominal white fat depots and supraclavicular brown fat in mice treated with (**A**,**B**) AAV9-GDF11 or (**C**,**D**) AAV9-GDF11ProFc-1 compared to control.