Figure 1

Glycosylation mutants (numbered 1-17; SEQ ID NOs: 2-18) aligned with wild type GDF15 (SEQ ID NO: 1)

```
          1         10         20         30         40         50
          |         |          |          |          |          |

WT        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
1         ARNGTHCPLGPGRCCRLHTVNASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
2         ARNGDHCPLGPGRCCNLTTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
3         ARNGDHCPLGPGRCCRLHTVRANLTDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
4         ARNGDHCPLGPGRCCRLHTVRASNETLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
5         ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPNQTRAANMHAQ 60
6         ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSNFTAANMHAQ 60
7         ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQNRTANMHAQ 60
8         ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFNATNMHAQ 60
9         ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
10        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
11        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
12        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
13        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
14        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
15        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
16        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
17        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60


          61        70         80         90         100        110
          |         |          |          |          |          |

WT        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:1)
1         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:2)
2         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:3)
3         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:4)
4         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:5)
5         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:6)
6         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:7)
7         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:8)
8         IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:9)
9         IKTNLTRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:10)
10        IKTSNHTLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:11)
11        IKTSLHRLKPDTVPAPCCVPANYTPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:12)
12        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQNTTTGVSLQTYDDLLAKDCHCI (SEQ ID NO:13)
13        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTNTTVSLQTYDDLLAKDCHCI (SEQ ID NO:14)
14        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDNGTSLQTYDDLLAKDCHCI (SEQ ID NO:15)
15        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGNSTQTYDDLLAKDCHCI (SEQ ID NO:16)
16        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVNLTTYDDLLAKDCHCI (SEQ ID NO:17)
17        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLNKTCHCI (SEQ ID NO:18)
```

Figure 2

Glycosylation ΔN3 mutants (numbered 18-34; SEQ ID NOs: 19-35) aligned with wild type GDF15 (SEQ ID NO: 1)

```
          1         10         20         30         40         50
          |         |          |          |          |          |

WT        ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
18           GTHCPLGPGRCCRLHTVNASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
19           GDHCPLGPGRCCNLTTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
20           GDHCPLGPGRCCRLHTVRANLTDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
21           GDHCPLGPGRCCRLHTVRASNETLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
22           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPNQTRAANMHAQ 60
23           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSNFTAANMHAQ 60
24           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQNRTANMHAQ 60
25           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFNATNMHAQ 60
26           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
27           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
28           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
29           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
30           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
31           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
32           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
33           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60
34           GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ 60


          61        70         80         90         100        110
          |         |          |          |          |          |

WT        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:1)
18        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:19)
19        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:20)
20        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:21)
21        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:22)
22        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:23)
23        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:24)
24        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:25)
25        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:26)
26        IKTNLTRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:27)
27        IKTSNHTLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:28)
28        IKTSLHRLKPDTVPAPCCVPANYTPMVLIQKTDTGVSLQTYDDLLAKDCHCI (SEQ ID NO:29)
29        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQNTTTGVSLQTYDDLLAKDCHCI (SEQ ID NO:30)
30        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTNTTVSLQTYDDLLAKDCHCI (SEQ ID NO:31)
31        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDNGTSLQTYDDLLAKDCHCI (SEQ ID NO:32)
32        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGNSTQTYDDLLAKDCHCI (SEQ ID NO:33)
33        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVNLTTYDDLLAKDCHCI (SEQ ID NO:34)
34        IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLNKTCHCI (SEQ ID NO:35)
```

Figure 3

M1 Fusion Protein: IgK Signal Sequence (lowercase) fused to HSA Amino Acid Sequence Fused with a $[(Gly_4\text{-}Ser)]_3$ linker (Underlined) to the N-Terminus of Mature Human GDF15 (Bolded) (SEQ ID NO: 77)

mdmrvpaqllgllllwlrgarcDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE
TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGER
AFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEK
SHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAA
ADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKH
PEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLS
EKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL<u>GGGGSGGGG
SGGGGS</u>**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPD
TVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI**

M2 Fusion Protein: IgK Signal Sequence (lowercase) fused to HSA Amino Acid Sequence Fused with a $[(Gly_4\text{-}Ser)]_3$ linker (Underlined) to the N-Terminus of Mature Human GDF15 (Bolded) containing a 3-amino acid (ΔARN) deletion (SEQ ID NO: 78)

mdmrvpaqllgllllwlrgarcDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE
TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGER
AFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEK
SHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAA
ADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKH
PEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLS
EKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL<u>GGGGSGGGG
SGGGGS</u>**GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVP
APCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI**

Figure 4

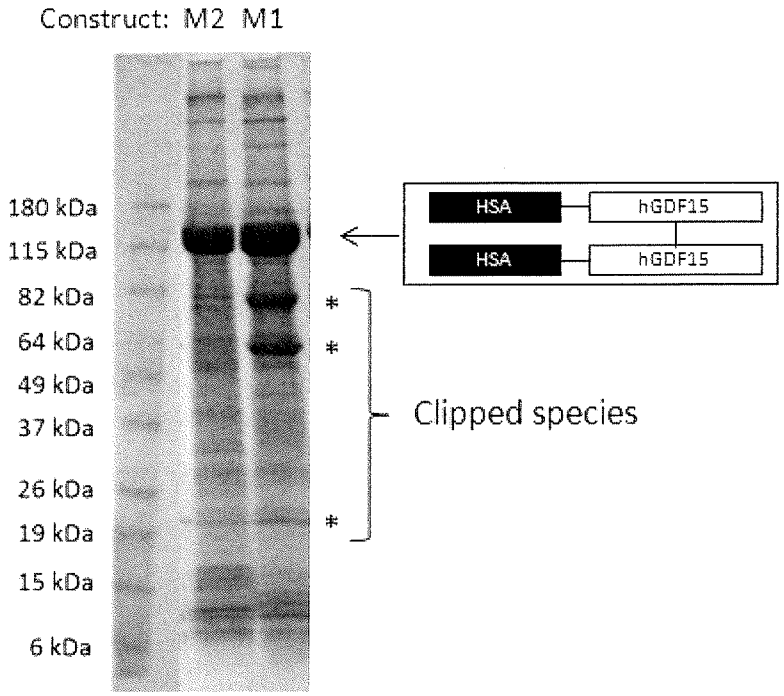

Construct: M2 M1
180 kDa
115 kDa
82 kDa
64 kDa
49 kDa
37 kDa
26 kDa
19 kDa
15 kDa
6 kDa
HSA
hGDF15
HSA
hGDF15
Clipped species

Figure 5

M3 Fusion Protein: IgK Signal Sequence (lowercase) fused to HSA Amino Acid Sequence Fused with a $[(Gly_4\text{-}Ser)]_5$ linker (Underlined) to the N-Terminus of Mature Human GDF15 (Bolded) Amino Acid Sequence containing a 3-amino acid deletion (denoted ΔARN or ΔN3) (SEQ ID NO: 79)

mdmrvpaqllgllllwlrgarcDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE
TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGER
AFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEK
SHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAA
ADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKH
PEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLS
EKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGG
SGGGGSGGGGSGGGGS**GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTS
LHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI**

M4 - Fusion Protein: IgK Signal Sequence (lowercase) fused to HSA Amino Acid Sequence Fused with a $[(Gly_4\text{-}Ser)]_5$ linker (Underlined) to the N-Terminus of Mature Human GDF15 (Bolded) Amino Acid Sequence containing a 6-amino acid deletion (denoted as ΔARNGDH or ΔN6) (SEQ ID NO: 80)

mdmrvpaqllgllllwlrgarcDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE
TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGER
AFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEK
SHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAA
ADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKH
PEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLS
EKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGG
SGGGGSGGGGSGGGGS**CPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHR
LKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI**

Figure 6

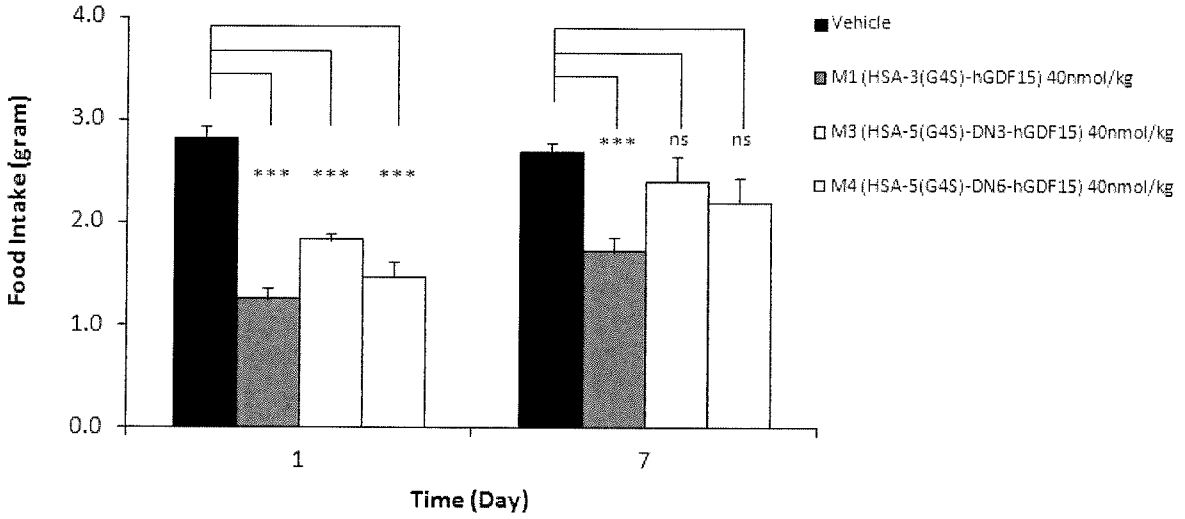


Figure 7

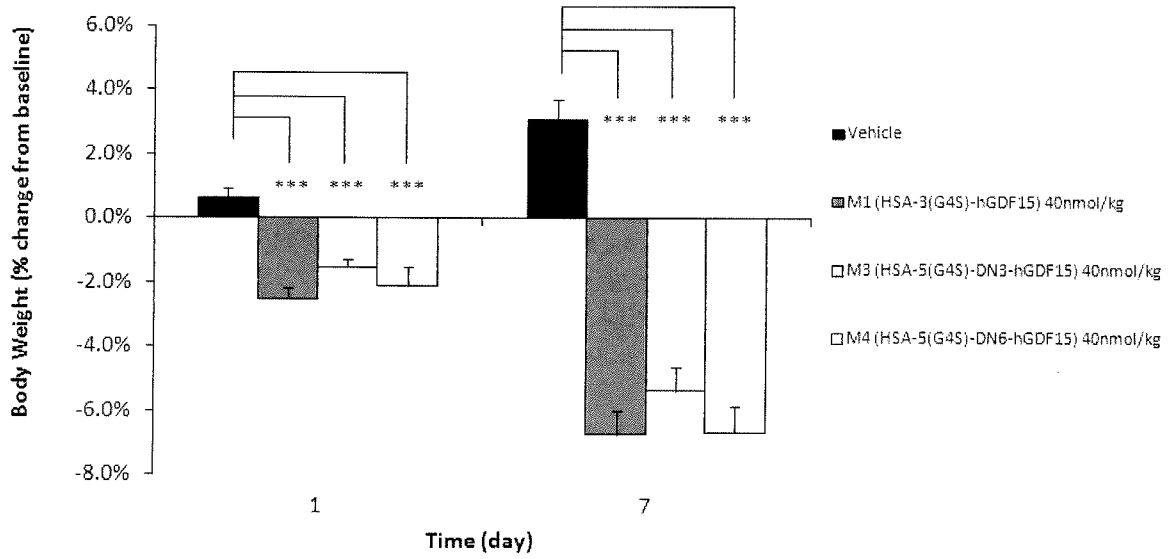


Figure 8A

hGDF15 Glycosylation muteins comprising an IgK Signal Sequence (lowercase) fused to the N-Terminus of Mature Human GDF15 (Bolded) Amino Acid Sequence

M5 - (SEQ ID NO: 81)
mdmrvpaqllgllllwlrgarc**ARNGTHCPLGPGRCCRLHTVNASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M6 - (SEQ ID NO: 82)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCNLTTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M7 - (SEQ ID NO: 83)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRANLTDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M8 - (SEQ ID NO: 84)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASNETLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M9 - (SEQ ID NO: 85)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PNQTRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M10 - (SEQ ID NO: 86)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSNFTAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M11 - (SEQ ID NO: 87)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQNRTANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M12 - (SEQ ID NO: 88)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFNATNMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M13 - (SEQ ID NO: 89)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTNLTRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M14 - (SEQ ID NO: 90)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSNHTLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

Figure 8A (continued)

M15 - (SEQ ID NO: 91)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPANYTPMVLIQKTDTGVSLQTYDDLLAKDCHCI

M16 - (SEQ ID NO: 92)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQNTTTGVSLQTYDDLLAKDCHCI

M17 - (SEQ ID NO: 93)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTNTTVSLQTYDDLLAKDCHCI

M18 - (SEQ ID NO: 94)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDNGTSLQTYDDLLAKDCHCI

M19 - (SEQ ID NO: 95)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGNSTQTYDDLLAKDCHCI

M20 - (SEQ ID NO: 96)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVNLTTYDDLLAKDCHCI

M21 - (SEQ ID NO: 97)
mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC**
PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLNKTCHCI

Figure 8B

Nucleic acid sequences encoding the hGDF15 Glycosylation muteins comprising an IgK Signal Sequence fused to the N-Terminus of Mature Human GDF15 Amino Acid Sequence (as shown in Figure 8A).

Nucleic acid sequences encoding M5

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGACTCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCAA
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 108)

Nucleic acid sequences encoding M6

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCAATCTGACCACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 109)

Nucleic acid sequences encoding M7

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGAACCTGACGGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 110)

Figure 8B (continued)

Nucleic acid sequences encoding M8

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGAATGAAACCCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 111)

Nucleic acid sequences encoding M9

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAACCAGACCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 112)

Nucleic acid sequences encoding M10

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCAACTTCACGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 113)

Nucleic acid sequences encoding M11

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGAACCGGACGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 114)

Figure 8B (continued)

Nucleic acid sequences encoding M12

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCAACGCGACGAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 115)

Nucleic acid sequences encoding M13

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
ACCTGACGCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 116)

Nucleic acid sequences encoding M14

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCAACCACACCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATA (SEQ ID NO: 117)

Nucleic acid sequences encoding M15

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAACTACACTCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 118)

Figure 8B (continued)

Nucleic acid sequences encoding M16

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAACACCACCACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 119)

Nucleic acid sequences encoding M17

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCAACACCACGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 120)

Nucleic acid sequences encoding M18

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACAACGGGACGTCGCTCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 121)

Nucleic acid sequences encoding M19

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGAACTCGACCCAGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 122)

Figure 8B (continued)

Nucleic acid sequences encoding M20

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG
CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC
ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA
GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC
CATGGTGCTCATTCAAAAGACCGACACCGGGGTGAACCTCACGACCTATGATGACTTGTTAGCC
AAAGACTGCCACTGCATATGA (SEQ ID NO: 123)

Nucleic acid sequences encoding M21

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat
gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCGCGCGTC
GCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACCATGTGCATCGGC
GCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGAGCCTGCACCGCCTGAAGC
CCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCGA
CACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAAACAAAACCTGCCACTGCATATGA (SEQ ID
NO: 124)

Figure 8C

ΔN3-M16 amino acid sequence (IgK Signal Sequence (lowercase) fused to the N-Terminus of Mature Human GDF15 (Bolded)

mdmrvpaqllgllllwlrgarc**GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQ FRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQNTTTGVSLQTYDDLLAKDCHCI** (SEQ ID NO: 100)

Nucleic acid sequence encoding ΔN3-M16

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat gtGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCGCGCGTCGCT GGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACCATGTGCATC GGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGAGCCTGCACC GCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCCCATGGTGCT CATTCAAAACACCACCACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCCAAAGACTGC CACTGCATATGA (SEQ ID NO: 125)

Figure 8D

Amino acid sequence of wild type mature human GDF15 fused to IgK signal sequence at the N-terminus (IgK-WT-GDF15)

mdmrvpaqllgllllwlrgarc**ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI** (SEQ ID NO: 126)

Nucleic acid encoding IgK-WT-GDF15

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagat gtGCGCGCAACGGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCG CGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACC ATGTGCATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGA GCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCC CATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCC AAAGACTGCCACTGCATATGA (SEQ ID NO: 127)

Figure 9

Human GDF15 Mutein Dimer Formation, N-Glycan Site Occupancy and Solubility assessment

Mutein ID	Dimer	N-Glyc	Solubility Inflection Point
hGDF15	YES	-	++
M5	YES	YES	++++
M6	YES	YES	Not Performed
M7	YES	YES	+++
M8	NO	NO	Not performed
M9	YES	YES	Not performed
M10	NO	NO	Not performed
M11	YES	YES	+++
M12	YES	YES	+++
M13	YES	YES	++++
M14	NO	NO	Not performed
M15	NO	NO	Not performed
M16	YES	YES	+++++
ΔN3-M16	YES	YES	+++++
M17	YES	YES	+++++
M18	YES	YES	Not performed
M19	YES	YES	Not performed
M20	YES	YES	++++
M21	YES	YES	+++

Figure 10

DIO Mice Model

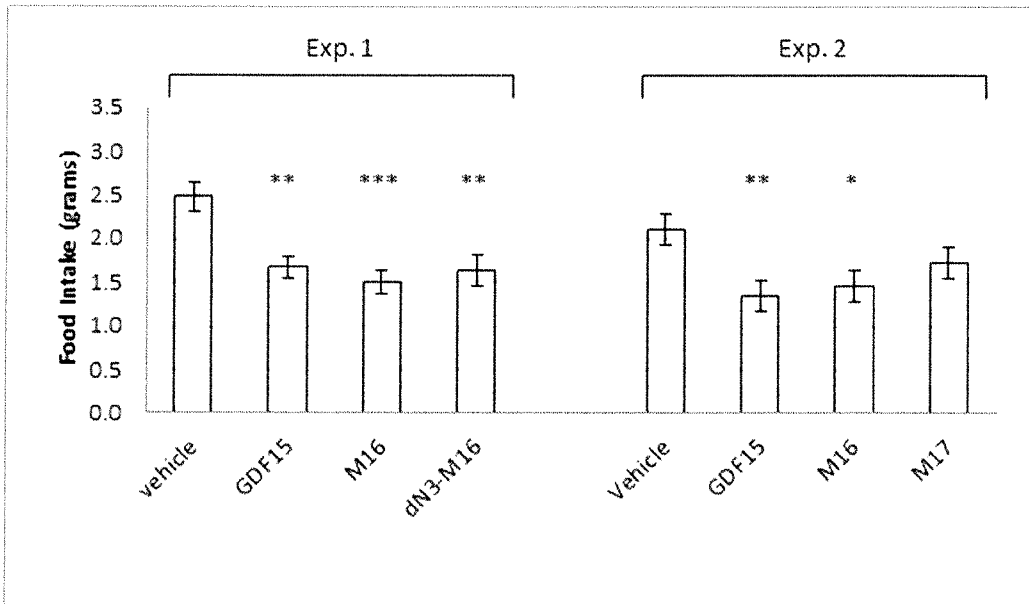


Figure 11

