

Design of knob-in-hole (Fc/Fc)-GDF15 molecules

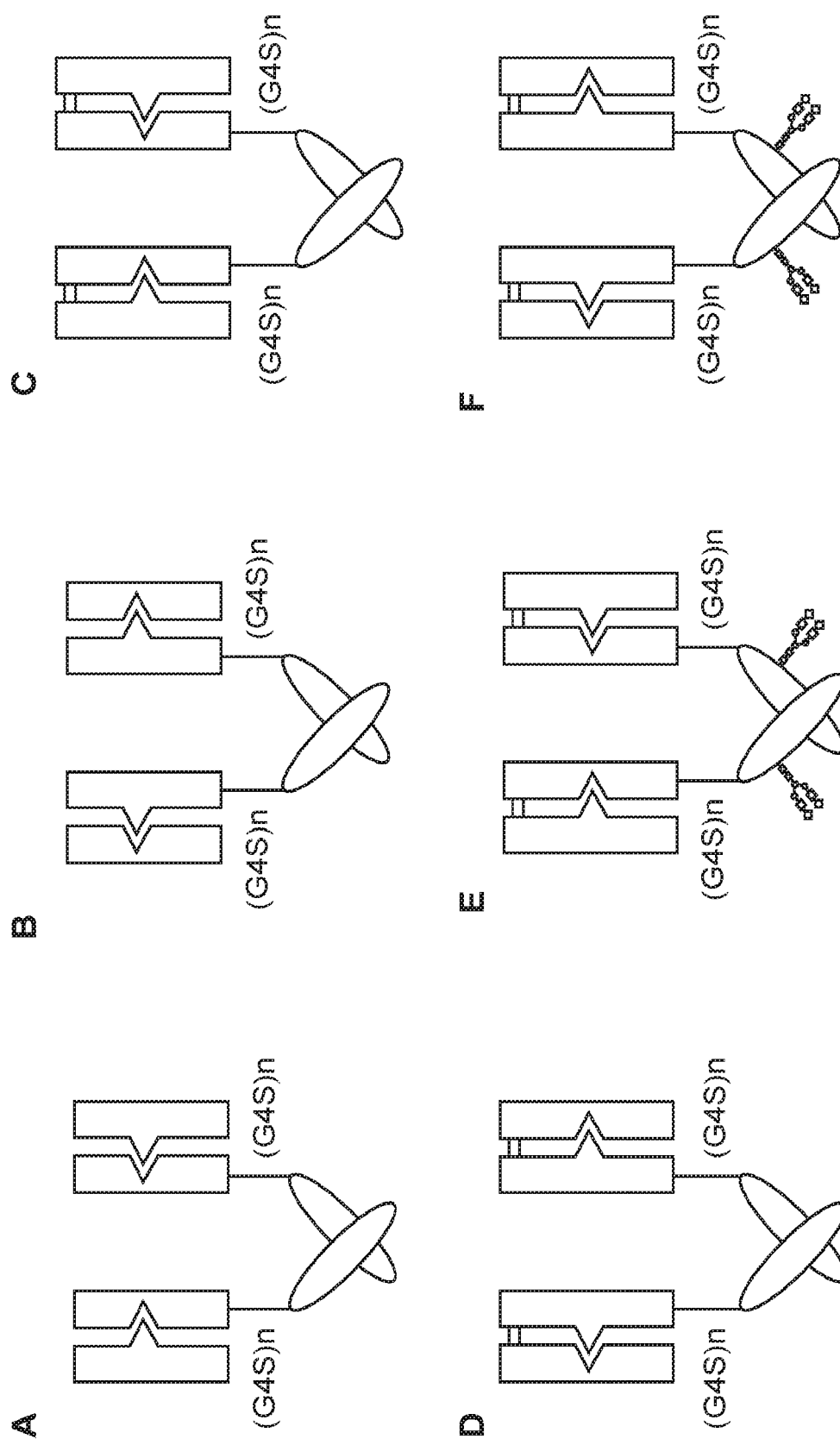


FIG. 1

Figure 2A – Recoveries of engineered knob-in-hole (Fc/Fc)-GDF15 molecules

<u>Fc-GDF15 chain A</u>	<u>Fc heterodimer (knob/hole) partner chain B</u>	<u>Variant number</u>	<u>GDF15 mutein (glycosylated)</u>	<u>Recovery (mg/L)</u>
hlgG1-Fc(AA)(T366Y)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(AA)(Y407T)	B1a/B1b	NO	<25
hlgG1-Fc(AA)(Y407T)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(AA)(T366Y)	B2a/B2b	NO	25-49.9
hlgG1-Fc(Δhinge, AA)(T366Y)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(Δhinge, AA)(Y407T)	B3a/B3b	NO	50-74.9
hlgG1-Fc(Δhinge, AA)(Y407T)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(Δhinge, AA)(T366Y)	B4a/B4b	NO	<25
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B5a/B5b	NO	<25
hlgG1-Fc(AA)(T366S, L368A, Y407V)-(G ₄ S) ₅ -ΔN6-GDF15 (C7-I112)	hlgG1-Fc(AA)(T366W)	B6a/B6b	NO	<25
hlgG1-Fc(Δhinge, AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)	hlgG1-Fc(Δhinge, AA)(T366S, L368A, Y407V)	B7a/B7b	NO	25-49.9
hlgG1-Fc(Δhinge, AA)(T366S, L368A, Y407V)-(G ₄ S) ₅ -ΔN6-GDF15(C7-I112)	hlgG1-Fc(Δhinge, AA)(T366W)	B8a/B8b	NO	N/A
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₃ -GDF15 (A1-I112) (D5T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B9a/B9b	YES	>100
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₄ -GDF15 (A1-I112) (D5T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B10a/B10b	YES	>100
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -GDF15 (A1-I112) (D5T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B11a/B11b	YES	>100
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₂ -ΔN2-GDF15 (N3-I112) (D5T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B12a/B12b	YES	75-99
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN2-GDF15 (N3-I112) (D5T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B13a/B13b	YES	>100
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)(R21N)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B14a/B14b	YES	75-99
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)(S23N/E25T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B15a/B15b	YES	75-99
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)(F52N/A54T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B16a/B16b	YES	>100
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)(R53N/A55T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B17a/B17b	YES	75-99
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112) (K91N/D93T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B18a/B18b	YES	75-99
hlgG1-Fc(AA)(T366W)-(G ₄ S) ₅ -ΔN3-GDF15 (G4-I112)(D93N/G95T)	hlgG1-Fc(AA)(T366S, L368A, Y407V)	B19a/B19b	YES	50-74.9

Figure 2B – Recoveries of GDF15 glycomutants

<u>Glycovariant</u>	<u>Recovery (mg/L)</u>
hGDF15 wild-type	< 0.99
R21N	< 0.99
R53N/A55T	4 - 7.99
S64N/H66T	16 - 31.99
P70N	2 - 3.99
Q90N	4 - 7.99
K91N/D93T	16 - 31.99
D93N/G95T	8 - 15.99
G95N	8 - 15.99
S97N/Q99T	8 - 15.99
L98N	4 - 7.99

Figure 3 – Reduction of body weight in DIO mouse model upon delivery of 0.4nmol/kg of (Fc/Fc)-GDF15 molecules

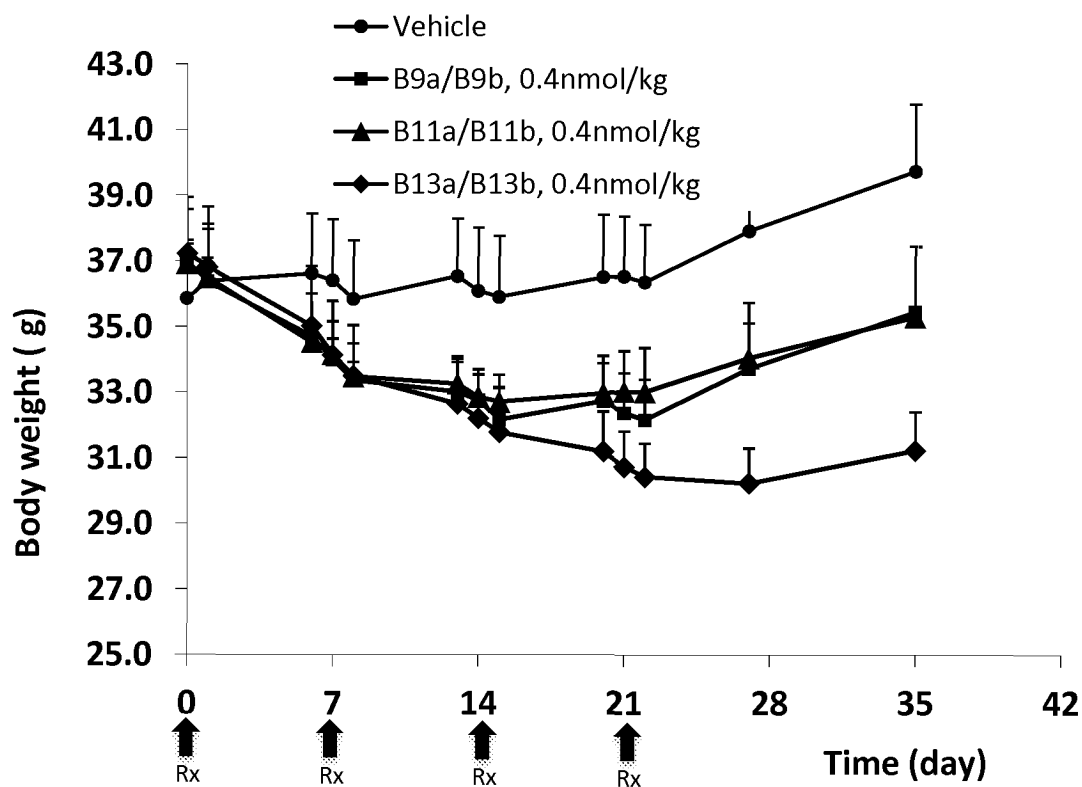


Figure 4 – Percent body weight Reduction in DIO mouse model upon delivery of 0.4nmol/kg of (Fc/Fc)-GDF15 molecules

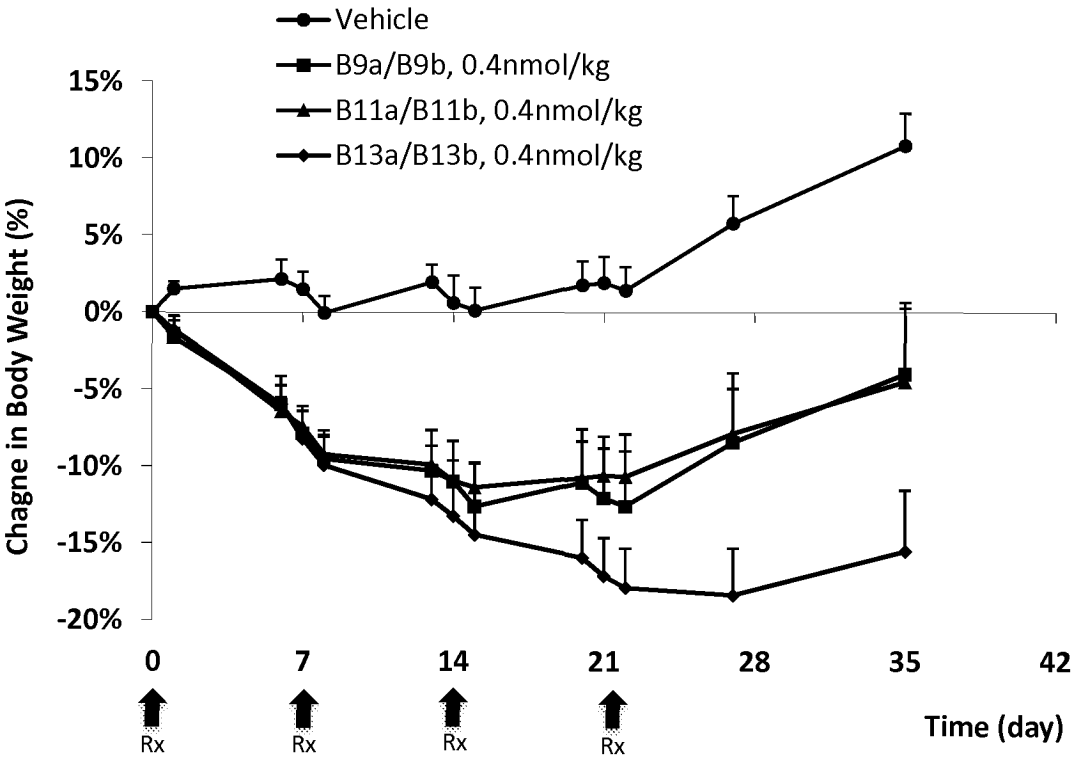


Figure 5 - Reduction of body weight in DIO mouse model upon delivery of 4.0nmol/kg of (Fc/Fc)-GDF15 molecules

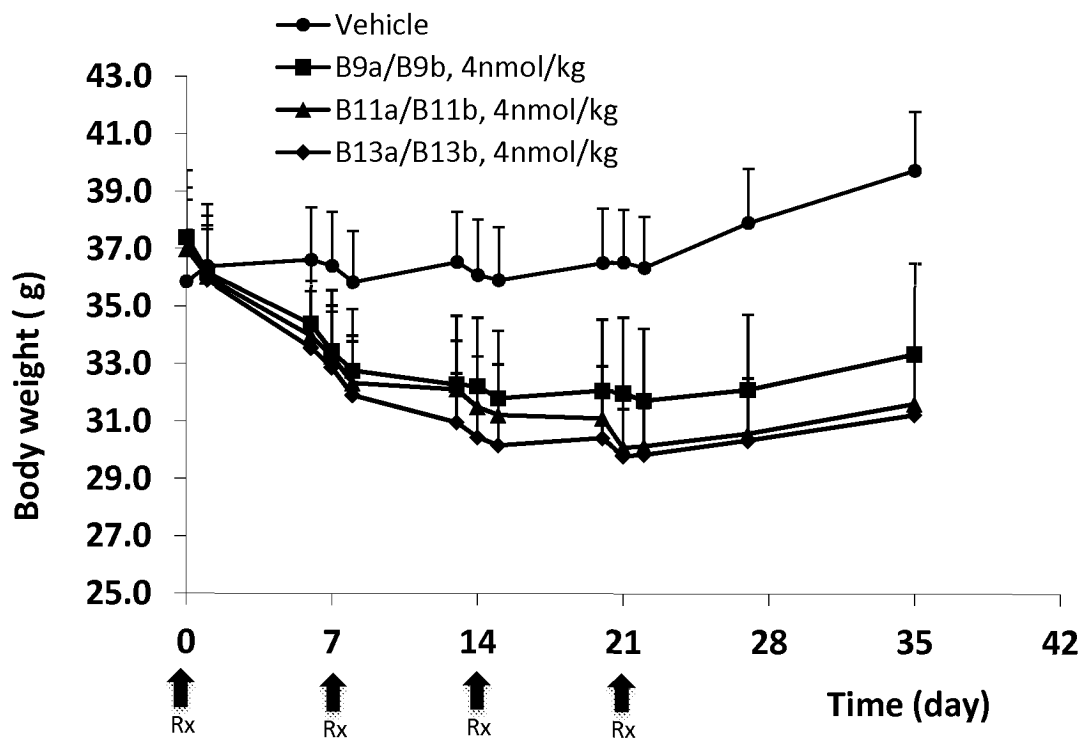


Figure 6 – Percent body weight Reduction in DIO mouse model upon delivery of 4.0nmol/kg of (Fc/Fc)-GDF15 molecules

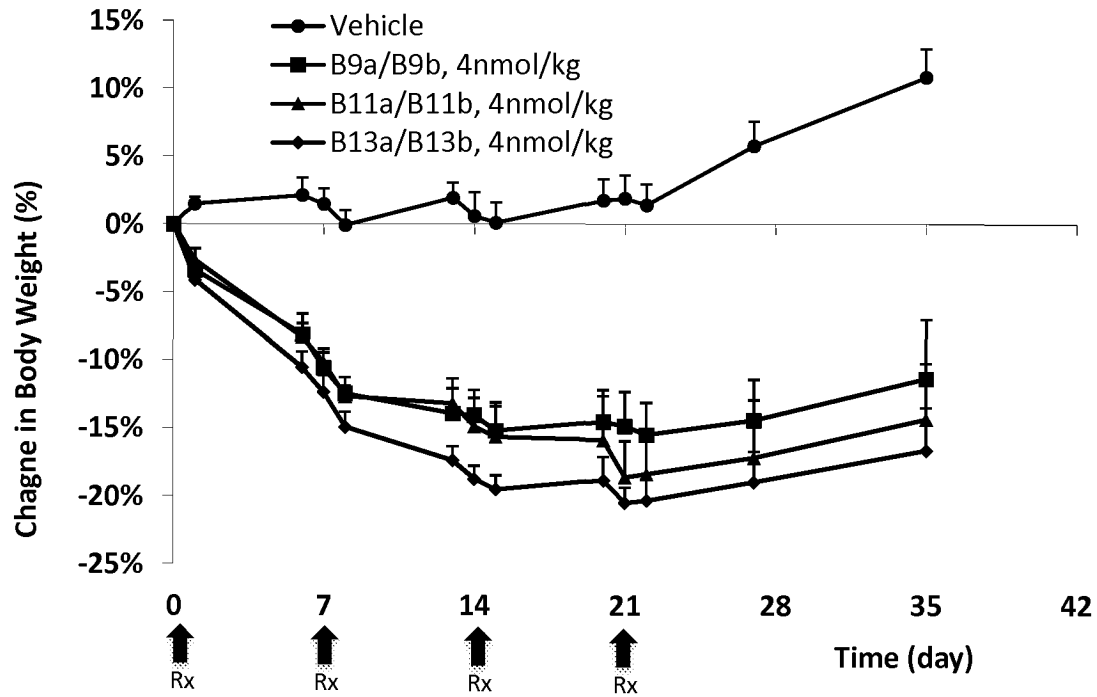


Figure 7 – Body weight (g) in DIO mouse for each time point for 0.4nmol/kg and 4.0nmol/kg of (Fc/Fc)-GDF15 molecules

	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	35.9	36.4	36.6	36.4	35.9	36.6	36.1	35.9	36.5	36.6	36.4	37.9	39.8
B9a/B9b 0.4nmol/kg	37.0	36.4	34.7	34.0	33.4	33.0	32.8	32.2	32.7	32.4	32.2	33.7	35.5
B9a/B9b 4nmol/kg	37.4	36.2	34.4	33.5	32.8	32.3	32.2	31.8	32.1	32.0	31.7	32.1	33.4
B11a/B11b 0.4nmol/kg	36.9	36.5	34.5	34.2	33.5	33.3	32.9	32.7	33.0	33.0	33.0	34.1	35.3
B11a/B11b 4nmol/kg	37.0	36.0	34.0	33.3	32.3	32.1	31.5	31.2	31.1	30.1	30.2	30.6	31.6
B13a/B13b 0.4nmol/kg	37.2	36.8	35.0	34.2	33.5	32.7	32.2	31.8	31.2	30.7	30.4	30.2	31.2
B13a/B13b 4nmol/kg	37.4	35.9	33.6	32.9	31.9	31.0	30.5	30.2	30.4	29.8	29.9	30.4	31.2

sem	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	1.77	1.72	1.81	1.87	1.78	1.74	1.92	1.84	1.89	1.82	1.76	1.87	2.04
B9a/B9b 0.4nmol/kg	1.58	1.58	1.30	1.17	1.13	0.91	0.93	0.99	1.16	1.21	1.23	1.39	2.01
B9a/B9b 4nmol/kg	2.32	2.35	2.27	2.12	2.14	2.39	2.37	2.35	2.47	2.63	2.51	2.61	3.15
B11a/B11b 0.4nmol/kg	0.59	0.58	0.52	0.47	0.45	0.77	0.69	0.82	1.15	1.23	1.35	1.69	2.15
B11a/B11b 4nmol/kg	1.70	1.78	1.89	1.76	1.65	1.69	1.76	1.76	1.81	1.72	1.68	1.93	1.87
B13a/B13b 0.4nmol/kg	1.69	1.80	1.81	1.64	1.55	1.44	1.33	1.36	1.21	1.09	1.02	1.07	1.17
B13a/B13b 4nmol/kg	1.70	1.75	1.96	1.94	1.87	1.70	1.66	1.69	1.71	1.64	1.63	1.71	1.97

Figure 8- Total body weight change (g) in DIO mouse model for each time point for 0.4nmol/kg and 4.0nmol/kg of (Fc/Fc)-GDF15 molecules

	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	0.0	0.5	0.8	0.5	0.0	0.7	0.2	0.0	0.6	0.7	0.5	2.1	3.9
B9a/B9b 0.4nmol/kg	0.0	-0.6	-2.3	-3.0	-3.6	-4.0	-4.2	-4.8	-4.3	-4.6	-4.8	-3.3	-1.5
B9a/B9b 4nmol/kg	0.0	-1.2	-3.0	-4.0	-4.6	-5.1	-5.2	-5.6	-5.3	-5.4	-5.7	-5.3	-4.1
B11a/B11b 0.4nmol/kg	0.0	-0.4	-2.4	-2.8	-3.4	-3.7	-4.1	-4.2	-4.0	-3.9	-3.9	-2.9	-1.6
B11a/B11b 4nmol/kg	0.0	-1.0	-3.0	-3.7	-4.7	-4.9	-5.5	-5.8	-5.9	-6.9	-6.8	-6.4	-5.4
B13a/B13b 0.4nmol/kg	0.0	-0.4	-2.2	-3.1	-3.7	-4.6	-5.0	-5.5	-6.0	-6.5	-6.8	-7.0	-6.0
B13a/B13b 4nmol/kg	0.0	-1.5	-3.9	-4.5	-5.5	-6.5	-7.0	-7.3	-7.0	-7.6	-7.6	-7.1	-6.2

sem	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	0.00	0.16	0.43	0.38	0.37	0.38	0.60	0.50	0.53	0.59	0.53	0.61	0.77
B9a/B9b 0.4nmol/kg	0.00	0.21	0.48	0.59	0.75	1.06	1.08	1.14	1.38	1.30	1.44	1.31	1.54
B9a/B9b 4nmol/kg	0.00	0.17	0.53	0.47	0.46	0.58	0.58	0.68	0.79	0.80	0.76	1.02	1.51
B11a/B11b 0.4nmol/kg	0.00	0.22	0.35	0.52	0.47	0.45	0.50	0.60	0.86	0.91	0.99	1.43	1.88
B11a/B11b 4nmol/kg	0.00	0.30	0.31	0.34	0.26	0.69	0.77	0.82	1.22	1.03	1.22	1.60	1.58
B13a/B13b 0.4nmol/kg	0.00	0.34	0.65	0.84	0.80	0.92	1.01	0.91	1.07	1.12	1.16	1.31	1.62
B13a/B13b 4nmol/kg	0.00	0.13	0.34	0.37	0.29	0.31	0.29	0.31	0.62	0.36	0.57	0.83	1.13

Unpaired t-test	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle													
B9a/B9b 0.4nmol/kg		**	***	***	**	**	**	**	**	**	**	**	*
B9a/B9b 4nmol/kg		***	***	***	***	***	***	***	***	***	***	***	***
B11a/B11b 0.4nmol/kg		**	***	***	***	***	***	***	**	**	**	**	*
B11a/B11b 4nmol/kg		**	***	***	***	***	***	***	***	***	***	***	***
B13a/B13b 0.4nmol/kg		*	**	**	**	***	**	***	***	***	***	***	***
B13a/B13b 4nmol/kg		***	***	***	***	***	***	***	***	***	***	***	***

Figure 9 - Percent Body weight reduction (%) in DIO mouse for each time point for 0.4nmol/kg and 4.0nmol/kg of (Fc/Fc)-GDF15 molecules

	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	0.0%	1.5%	2.2%	1.5%	0.0%	2.0%	0.6%	0.1%	1.8%	1.9%	1.4%	5.8%	10.8%
B9a/B9b 0.4nmol/kg	0.0%	-1.6%	-6.0%	-7.9%	-9.5%	-10.3%	-11.0%	-12.6%	-11.0%	-12.1%	-12.6%	-8.4%	-4.0%
B9a/B9b 4nmol/kg	0.0%	-3.3%	-8.1%	-10.6%	-12.4%	-13.9%	-14.1%	-15.2%	-14.5%	-14.8%	-15.5%	-14.4%	-11.4%
B11a/B11b 0.4nmol/kg	0.0%	-1.1%	-6.4%	-7.4%	-9.2%	-9.9%	-11.0%	-11.4%	-10.8%	-10.6%	-10.7%	-7.9%	-4.5%
B11a/B11b 4nmol/kg	0.0%	-2.6%	-8.3%	-10.2%	-12.6%	-13.2%	-14.9%	-15.6%	-15.9%	-18.6%	-18.4%	-17.2%	-14.4%
B13a/B13b 0.4nmol/kg	0.0%	-1.1%	-6.0%	-8.2%	-9.9%	-12.2%	-13.2%	-14.5%	-15.9%	-17.1%	-17.9%	-18.4%	-15.5%
B13a/B13b 4nmol/kg	0.0%	-4.1%	-10.5%	-12.3%	-14.9%	-17.4%	-18.8%	-19.5%	-18.8%	-20.5%	-20.3%	-19.0%	-16.7%

sem	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle	0.00%	0.48%	1.27%	1.14%	1.10%	1.10%	1.77%	1.48%	1.56%	1.69%	1.52%	1.77%	2.09%
B9a/B9b 0.4nmol/kg	0.00%	0.60%	1.25%	1.45%	1.86%	2.65%	2.63%	2.77%	3.46%	3.24%	3.58%	3.49%	4.29%
B9a/B9b 4nmol/kg	0.00%	0.58%	1.53%	1.13%	1.19%	1.85%	1.86%	2.09%	2.36%	2.52%	2.35%	3.01%	4.37%
B11a/B11b 0.4nmol/kg	0.00%	0.59%	0.89%	1.29%	1.16%	1.23%	1.32%	1.63%	2.38%	2.54%	2.77%	3.94%	5.16%
B11a/B11b 4nmol/kg	0.00%	0.86%	1.00%	1.00%	0.76%	1.83%	2.09%	2.25%	3.23%	2.68%	3.13%	4.24%	4.09%
B13a/B13b 0.4nmol/kg	0.00%	0.92%	1.82%	2.12%	1.98%	2.22%	2.35%	2.14%	2.46%	2.46%	2.55%	3.04%	3.97%
B13a/B13b 4nmol/kg	0.00%	0.44%	1.15%	1.22%	1.11%	1.03%	0.96%	1.04%	1.72%	1.13%	1.57%	2.24%	3.15%

Unpaired t-test

	D 0	D1	D6	D7	D8	D13	D14	D15	D20	D21	D22	D27	D35
Vehicle													
B9a/B9b 0.4nmol/kg		**	***	***	**	**	**	**	**	**	**	**	*
B9a/B9b 4nmol/kg		***	***	***	***	***	***	***	***	***	***	***	***
B11a/B11b 0.4nmol/kg		**	***	***	***	***	***	***	***	***	**	*	*
B11a/B11b 4nmol/kg		**	***	***	***	***	***	***	***	***	***	***	***
B13a/B13b 0.4nmol/kg		*	**	**	**	***	***	***	***	***	***	***	***
B13a/B13b 4nmol/kg		***	***	***	***	***	***	***	***	***	***	***	***