

FIGURES

Figure 1:

K_D (nM)	hIGF1-Ea-Fc_mut_13/2_A	hIGF-1
IGF-1R	373	204

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Figure 2:

A

ligand	EC ₅₀ nM ± SEM
hIGF-1	1.8 ± 0.1
hIGF1-Ea-Fc_mut_02	2.7*
hIGF1-Ea-Fc_mut_03	9.8**
hIGF1-Ea-Fc_mut_04	3.04*
hIGF1-Ea-Fc_mut_12	3.81*
hIGF1-Ea-Fc_mut_13	4.17*
hIGF1-Ea-D1-3, R37A, D71-72, R77Q-Fc	5.26 ± 0.7#

*n=2; **n=1; #P<0.0001 versus hIGF-1, t-test

B

ligand	EC ₅₀ nM ± SEM
hIGF-1	1.8 ± 0.1
hIGF1-Ea-Fc_mut_04/2_E	7.4 ± 1.3#
hIGF1-Ea-Fc_mut_04_E	5.5*
hIGF1-Ea-Fc_mut_13/2_A	5.7*
hIGF1-Ea-Fc_mut_13_A	4.1*

*n=2; #P<0.0001 versus hIGF-1, t-test

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C

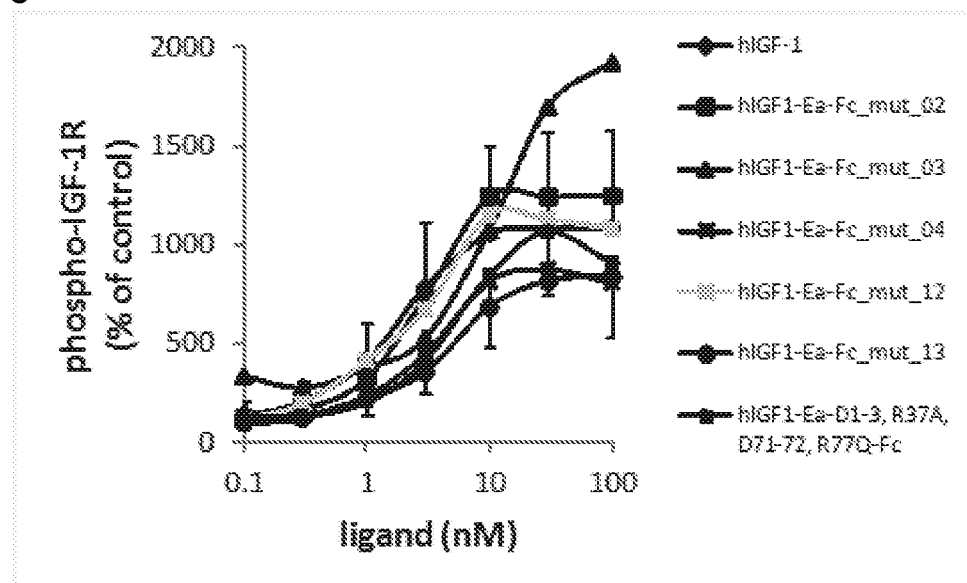
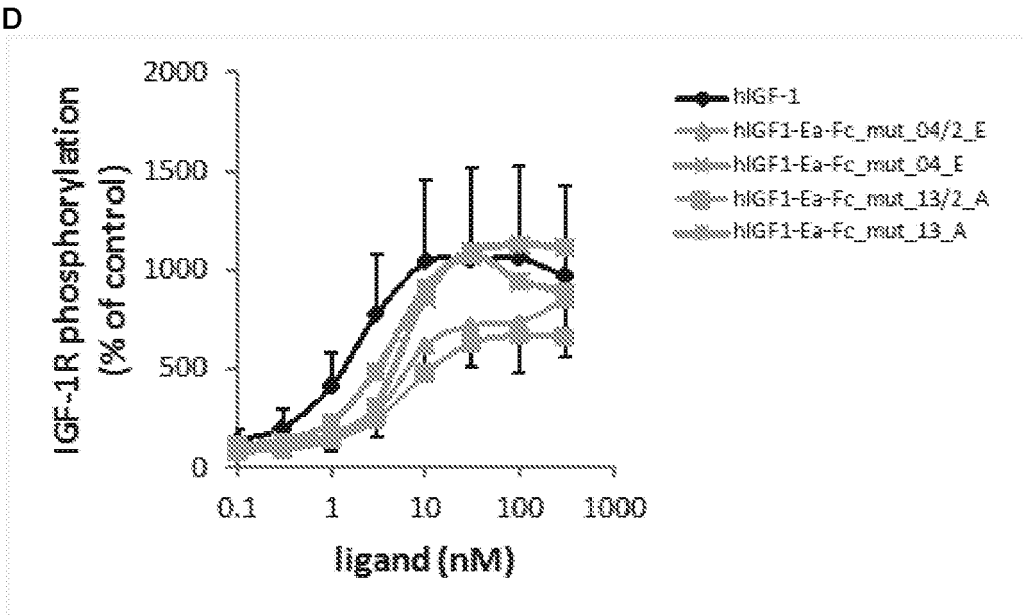


Figure 2 continued:



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Figure 3:

5 A

ligand	Mean max fold increase versus control $\pm$ SD
Insulin	20.8 $\pm$ 6.1
hIGF-1	3.9 $\pm$ 0.8 [#]
hIGF1-Ea-Fc_mut_02	5.9 $\pm$ 1.1 ^{#,\$}
hIGF1-Ea-Fc_mut_03	8.6 [*]
hIGF1-Ea-Fc_mut_04	12.5 [*]
hIGF1-Ea-Fc_mut_12	21.9 [*]
hIGF1-Ea-Fc_mut_13	15.2 [*]
hIGF1-Ea-D1-3, R37A, D71-72, R77Q- Fc	18.8 $\pm$ 3.4

*n=2; [#]P<0.001 versus insulin; ^{\$}P<0.05 versus IGF-1

B

ligand	Mean max fold increase versus control $\pm$ SD
Insulin	20.8 $\pm$ 6.1
hIGF-1	3.9 $\pm$ 0.8 [#]
hIGF1-Ea-Fc_mut_04/2_E	5.2 [*]
hIGF1-Ea-Fc_mut_04_E	9.4 [*]
hIGF1-Ea-Fc_mut_13/2_A	5.4 [*]
hIGF1-Ea-Fc_mut_13_A	12.4 [*]

*n=2; ; [#]P<0.001 versus insulin

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Figure 3 continued:

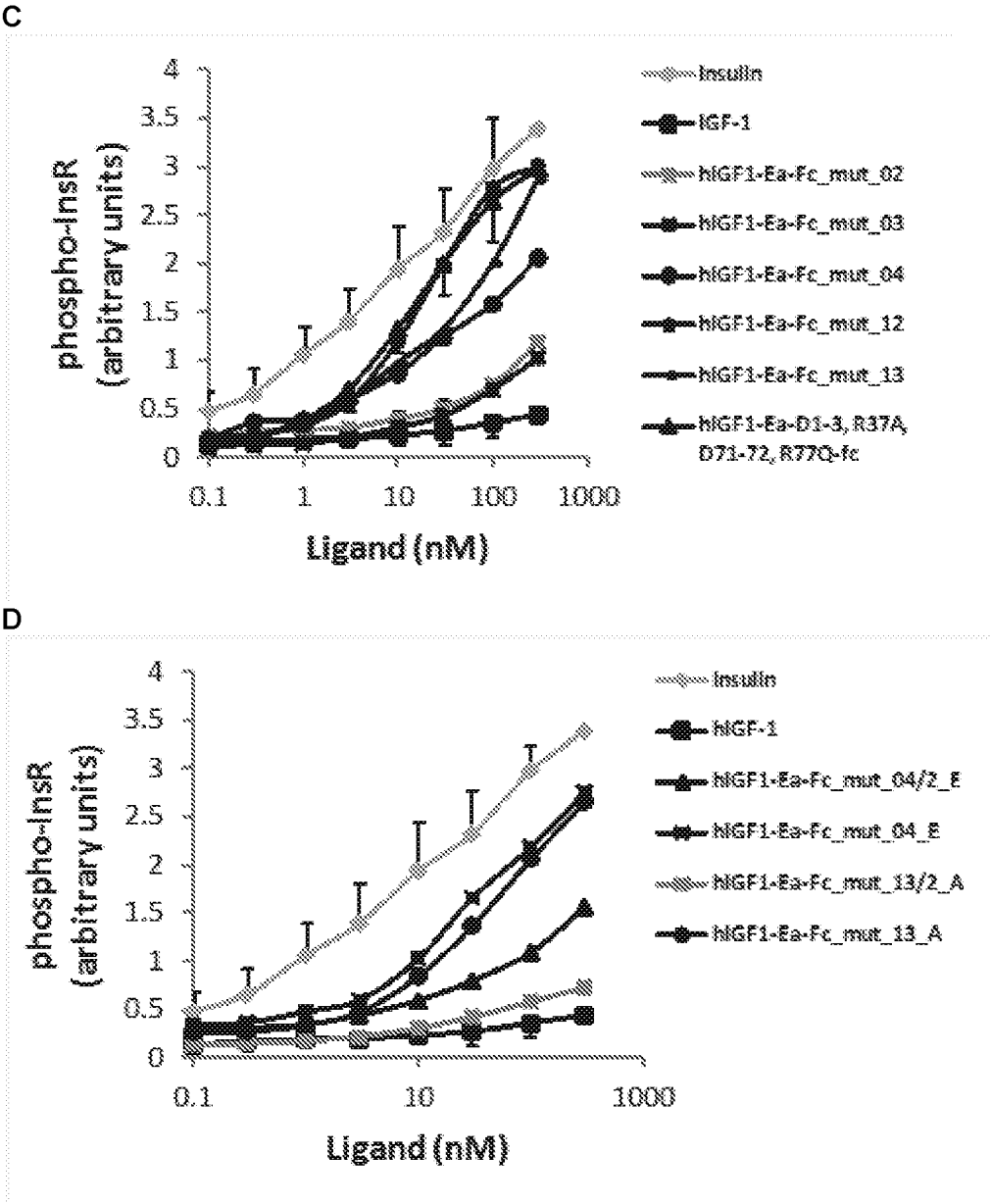
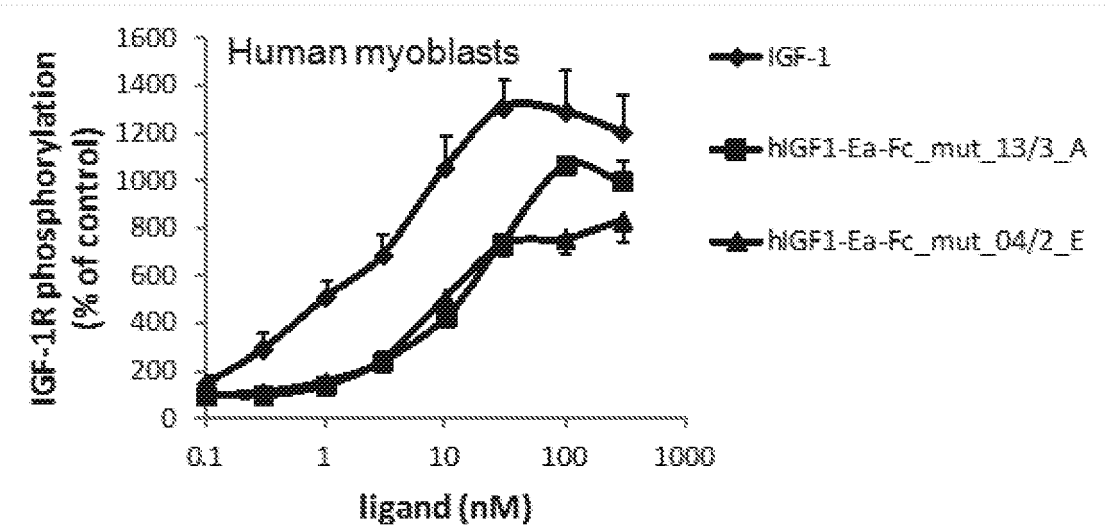


Figure 4:
A

Peptide	Human myoblasts		Cynomolgus myoblasts	
	EC ₅₀ (nM) ± SEM	E _{max} (% of control) ± SEM	EC ₅₀ (nM) ± SEM	E _{max} (% of control) ± SEM
hIGF-1	4.1 ± 1.4	1309 ± 119	5.1 ± 0.7	786 ± 182
hIGF1-Ea-Fc_mut_13/2_A	16.8 ± 3.1*	1069 ± 28	25.4 ± 4.0#	779 ± 145
hIGF1-Ea-Fc_mut_04/2_E	10.6 ± 2.4	834 ± 83*	23.8 ± 2.0#	724 ± 152

**P* < 0.05 vs Human myoblasts treated with hIGF-1. #*P* < 0.05 vs Cynomolgus myoblasts treated with hIGF-1. ANOVA, Tukey's test

B



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C

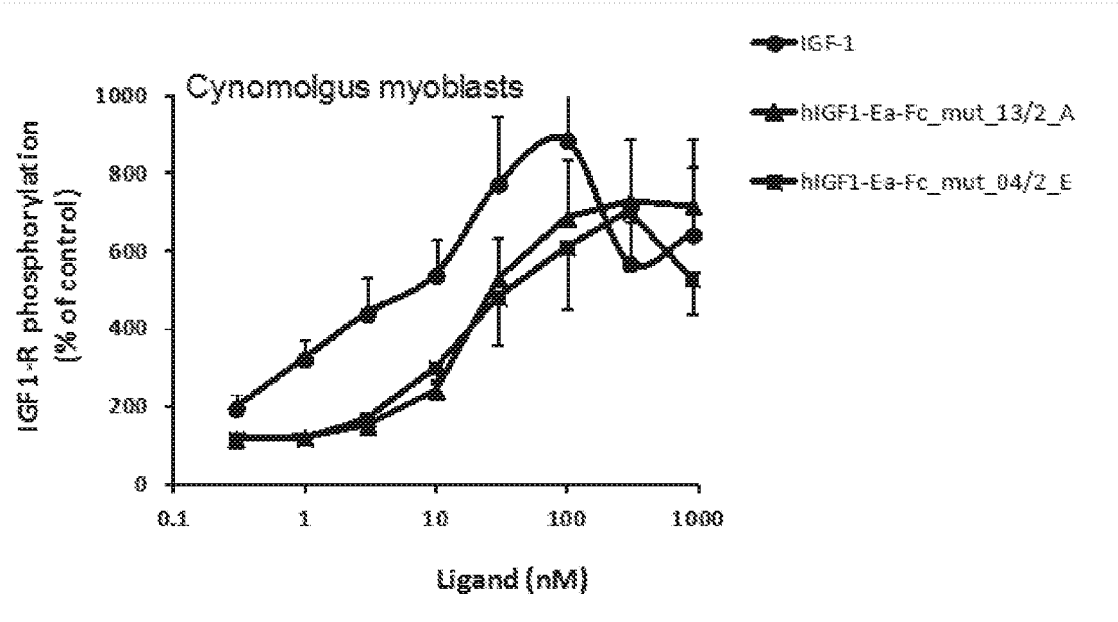


Figure 5:

A

ligand	EC50 (nM) $\pm$ SD	max fold increase of control $\pm$ SD
Insulin	11.5 $\pm$ 5.6**	1.37 $\pm$ 0.08
hIGF-1	1.3 $\pm$ 0.9	1.32 $\pm$ 0.06
hIGF1-Ea Fc_mut_13/2_A	4.3 $\pm$ 2.0	1.31 $\pm$ 0.11
hIGF1-Ea Fc_mut_04/2_E	3.7 $\pm$ 1.1	1.37 $\pm$ 0.10

**P < 0.001 versus IGF-1, ANOVA, Tukey's test

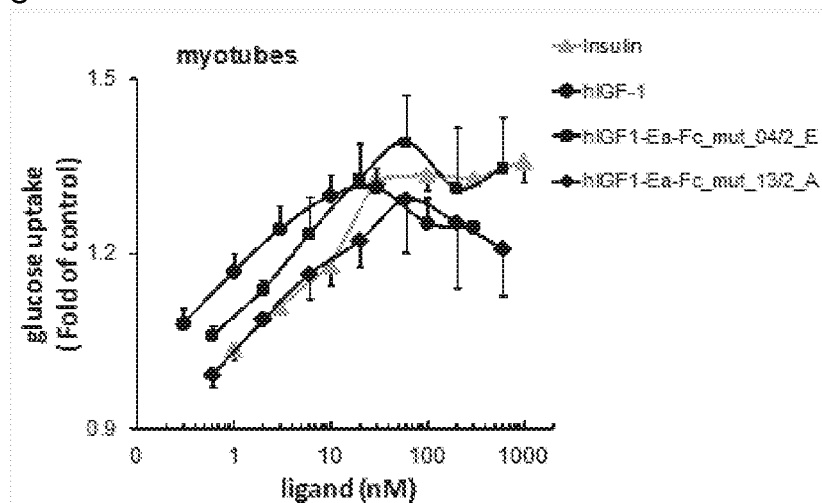
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B

ligand	EC50 (nM) $\pm$ SD	max fold increase of control $\pm$ SD
Insulin	9.1 $\pm$ 3.7	5.2 $\pm$ 1.00
hIGF-1	5.0 $\pm$ 1.4	4.6 $\pm$ 0.68
hIGF1-Ea Fc_mut_13/2_A	49.9 $\pm$ 4.6**,***	3.2 $\pm$ 0.25#
hIGF1-Ea Fc_mut_04/2_E	49.0 $\pm$ 28.4**,***	4.0 $\pm$ 0.47

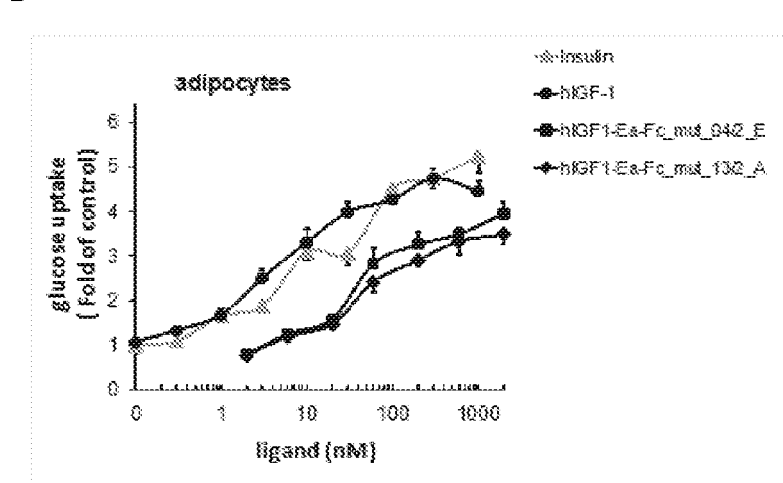
P < 0.05 and **P < 0.01 versus Insulin; ***P < 0.001 versus IGF-1 ANOVA, Tukey's test

C



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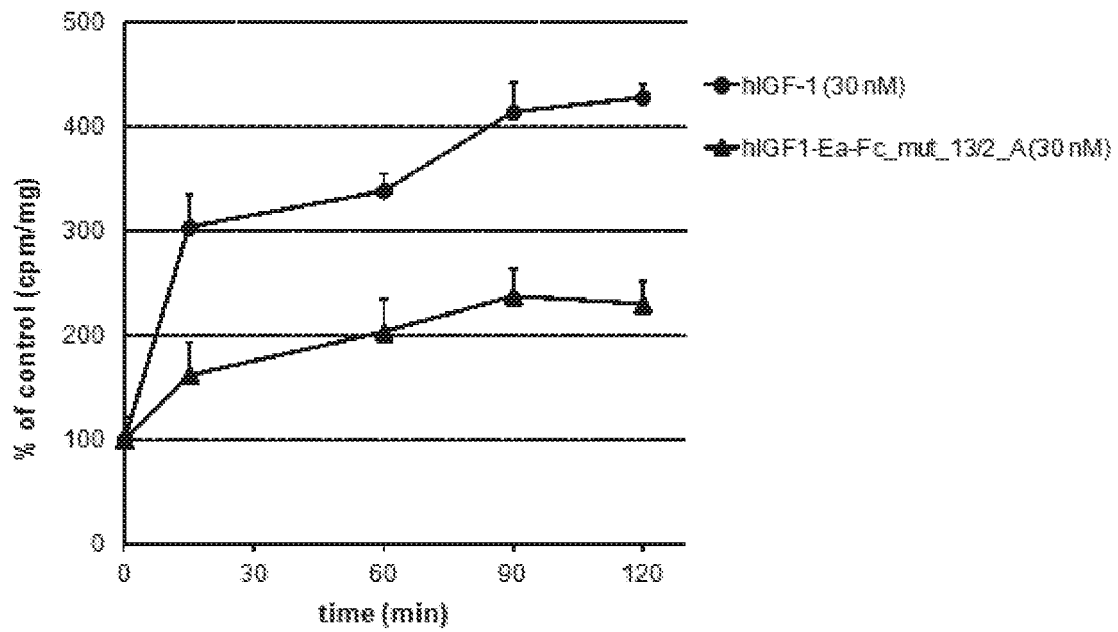
D



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Figure 6:



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Figure 7:

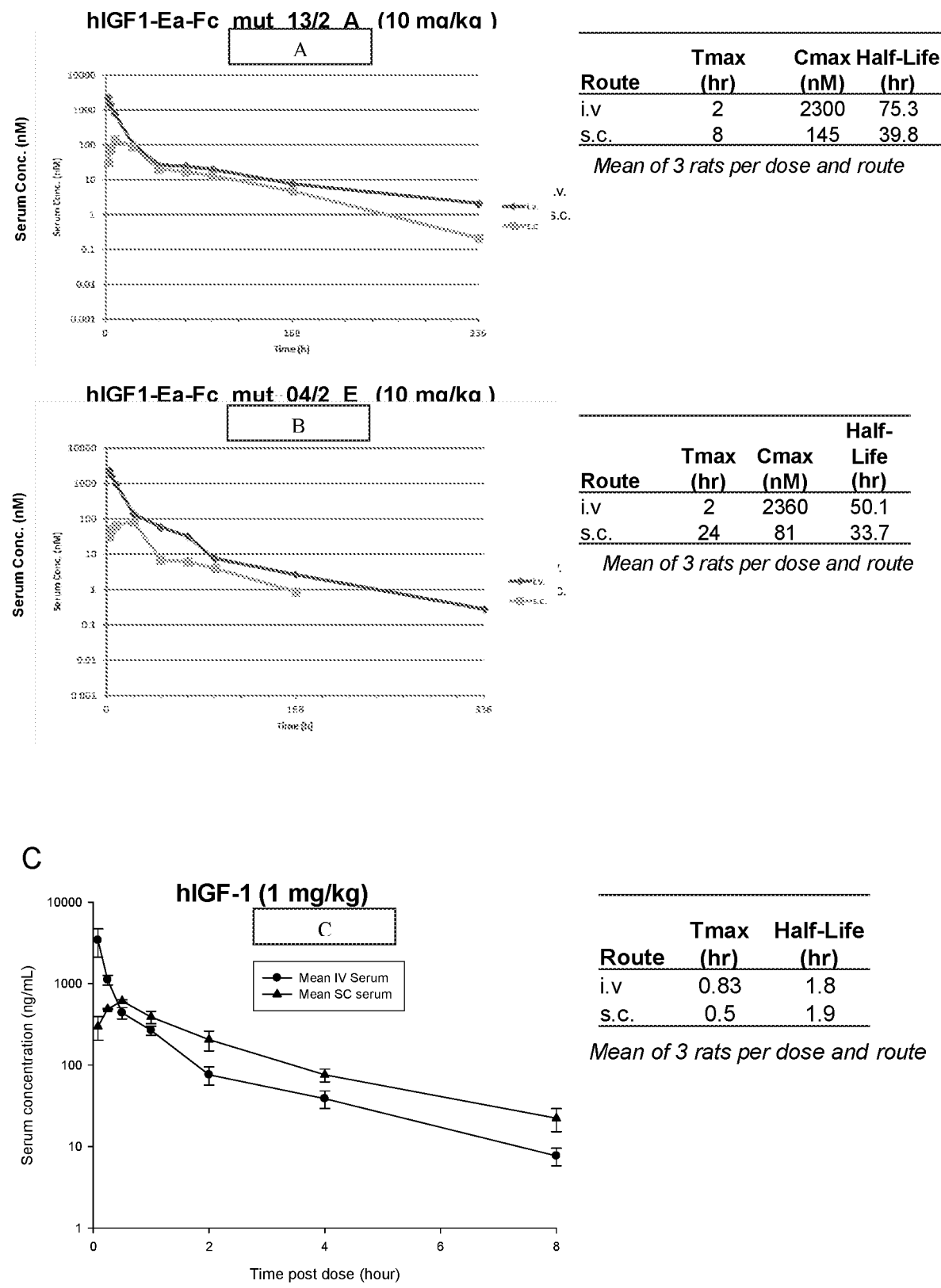


Figure 8:

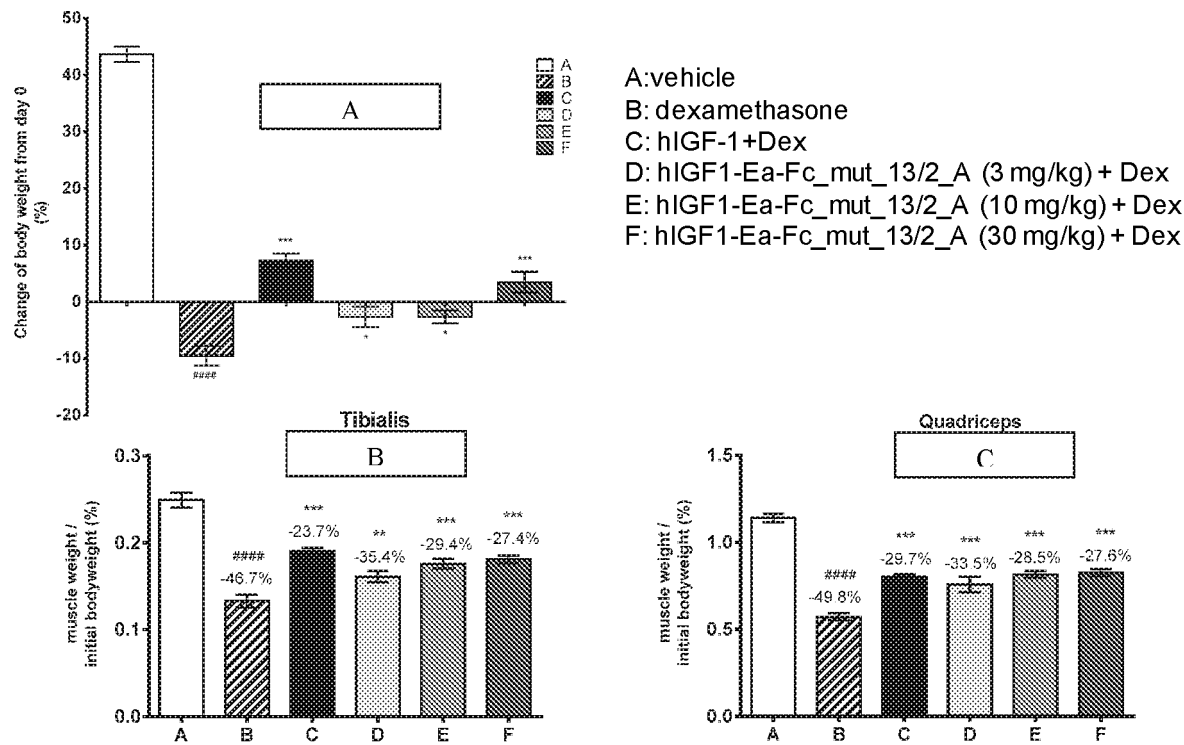


Figure 9:

Name	Titer in supernatant (mg/l)	concentration (mg/ml)	Aggregates (%)
hIGF1-Ea-D1-3, R36Q, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.:78)	33	0.88	37.0
hlgF1-Ea-Fc_mut 13/2_A: SEQ ID NO.: 9	83.1	1.65	9.8
hIGF1-Ea-D1-3, R36Q, G42A, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 79)	11.79	0.49	37.4
hIGF1-Ea-D1-3, R36Q, G42Q, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.:80)	16.98	0.61	43.2
hIGF1-Ea-D1-3, R36Q, G42P, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 81)	18.57	0.54	71.9
hIGF1-Ea-D1-3, R36Q, G42K, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.:82)	21.6	0.56	53.8
hIGF1-Ea-D1-3, R36Q, G42E, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 83)	26.97	0.84	49.4
hIGF1-Ea-D1-3, R36Q, G42I, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 84)	8.7	0.4	85.5
hIGF1-Ea-D1-3, R36Q, G42Y, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 85)	13.38	0.41	48.5
hIGF1-Ea-D1-3, R36Q, D42, D68-72, R74Q, R77Q, R104Q -fc domain (SEQ ID NO.: 86)	78.6	1.96	21.0

D: deletion; fc domain: the Fc portion from hlgG1

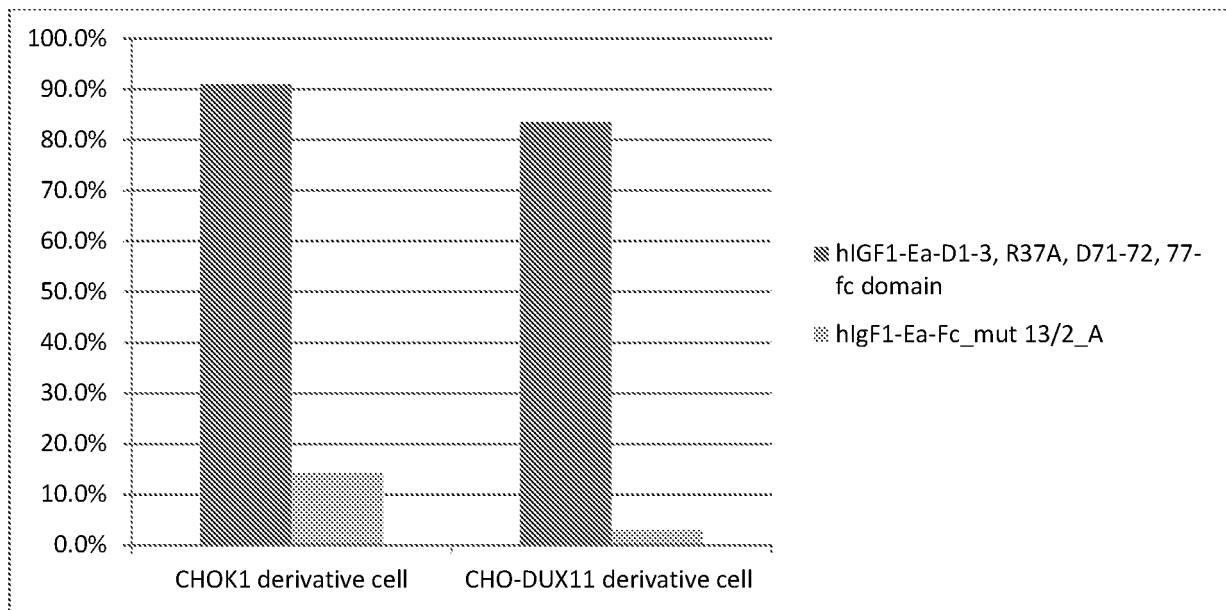
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Figure 10:



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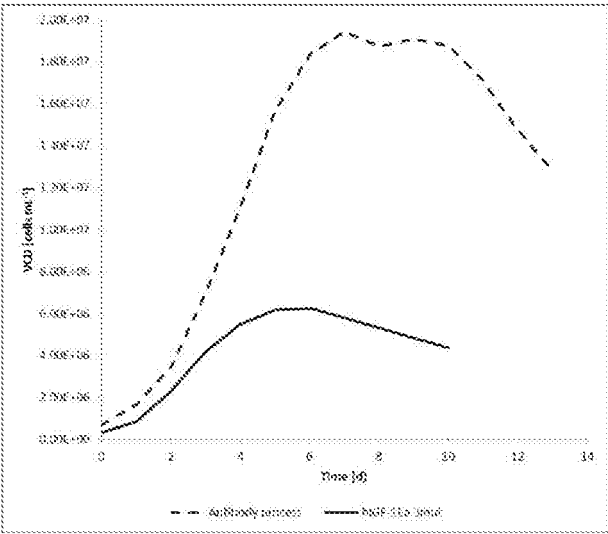
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Figure 11
A)



B)

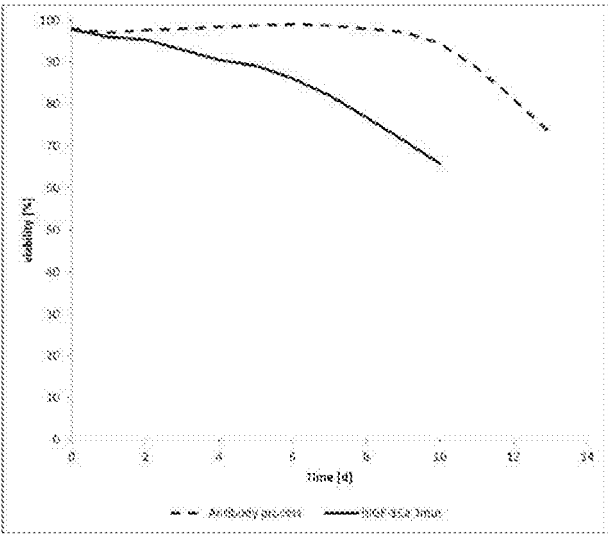
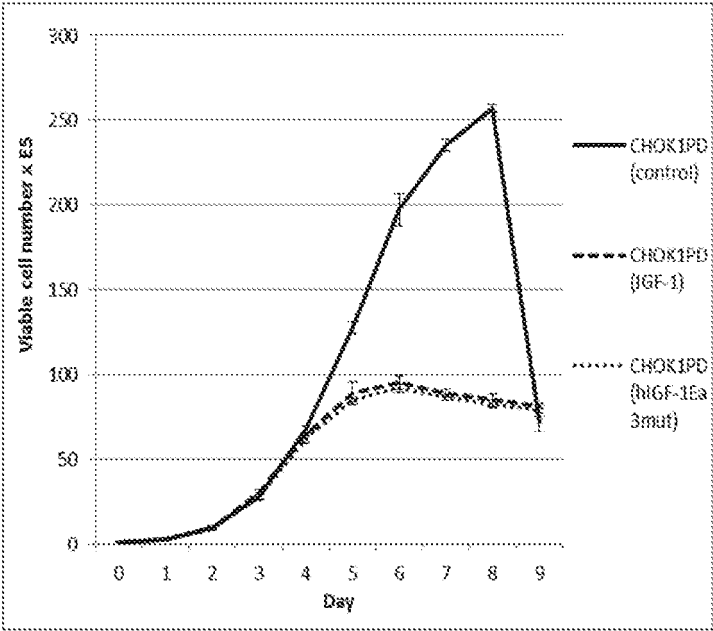


Figure 12
A)



B)

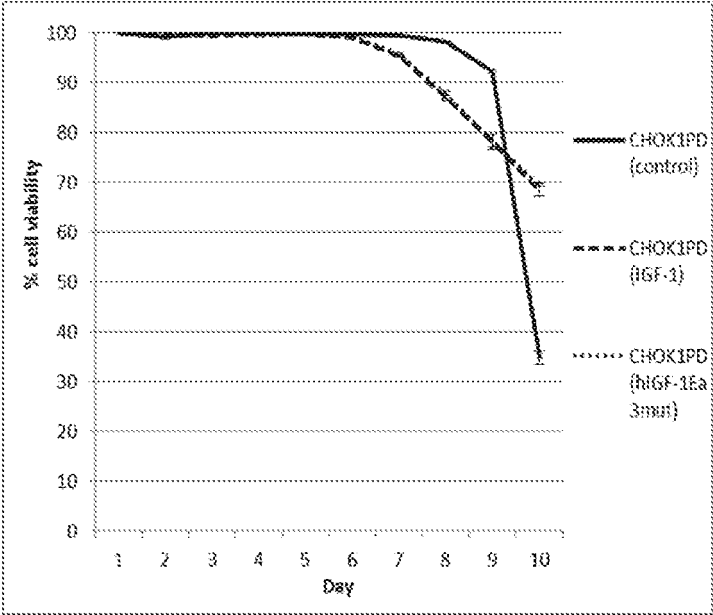
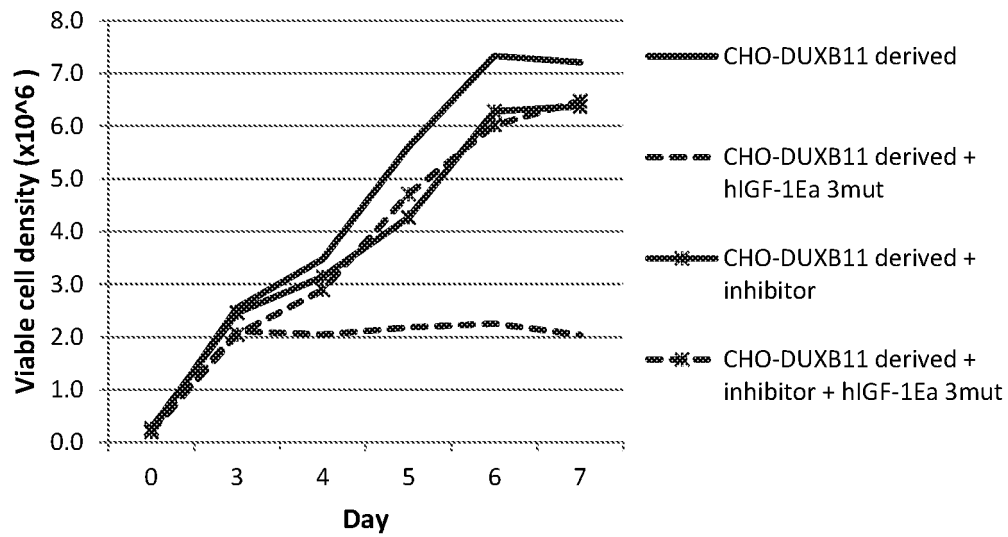


Figure 13



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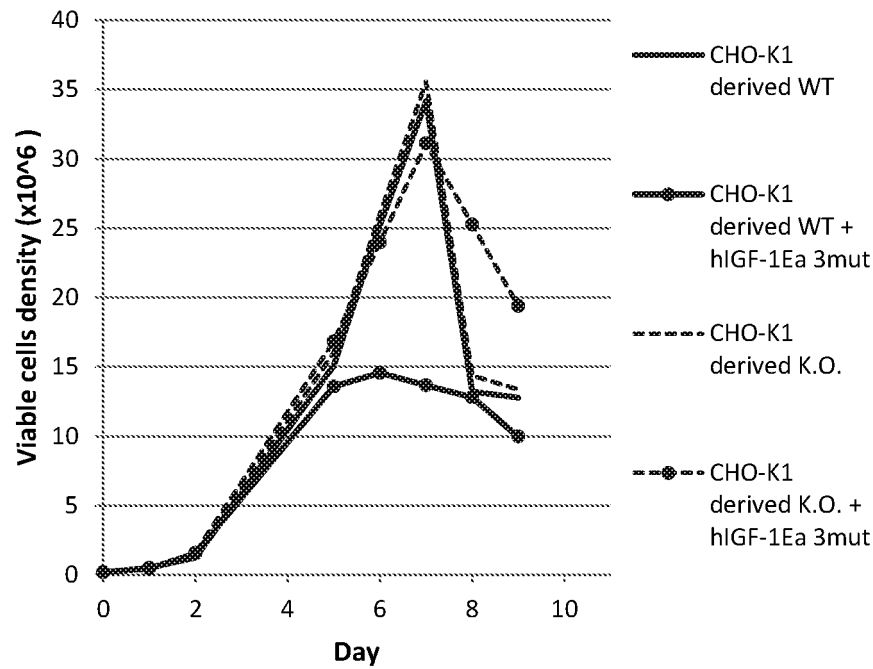
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Figure 14

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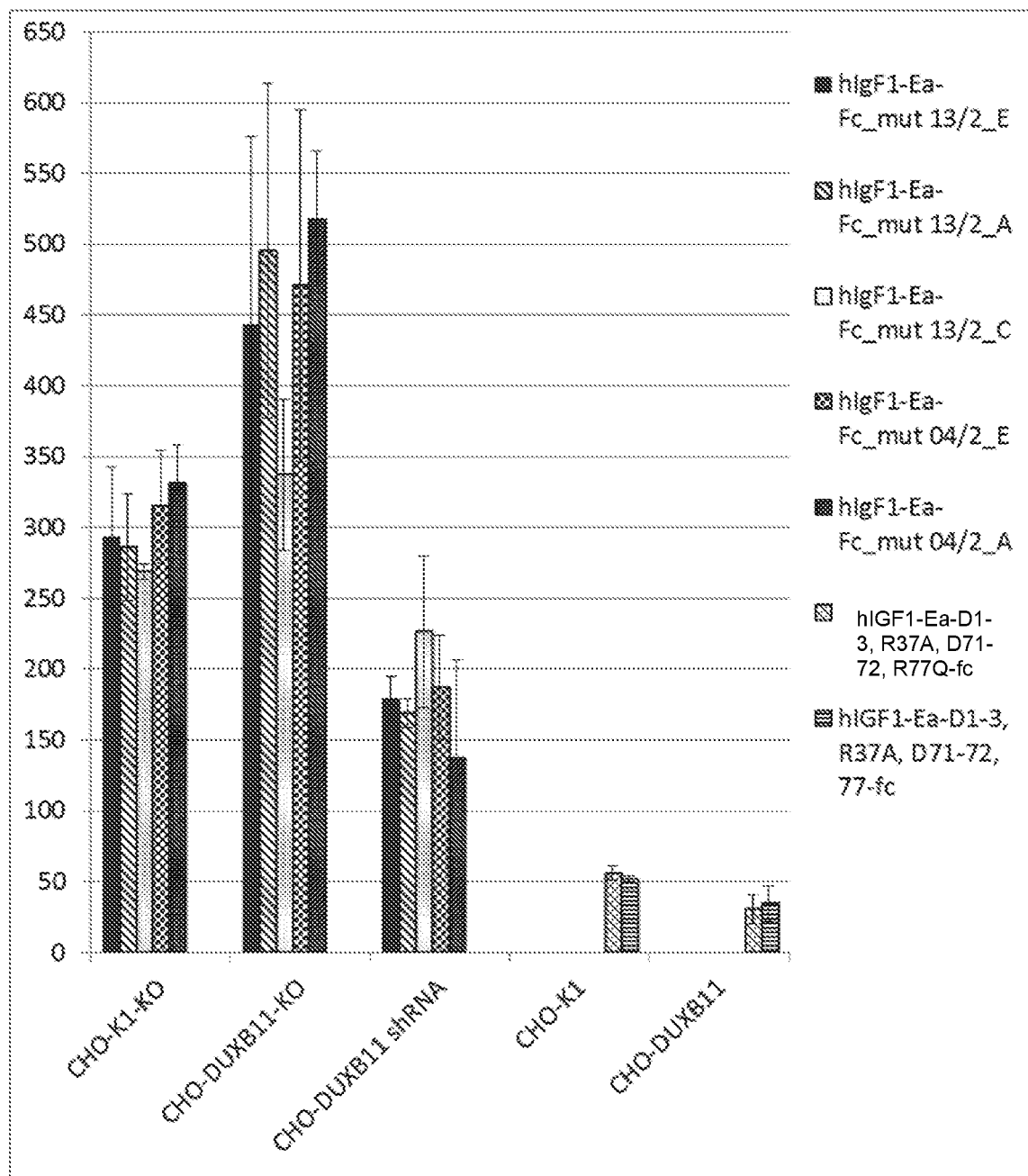
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Figure 15

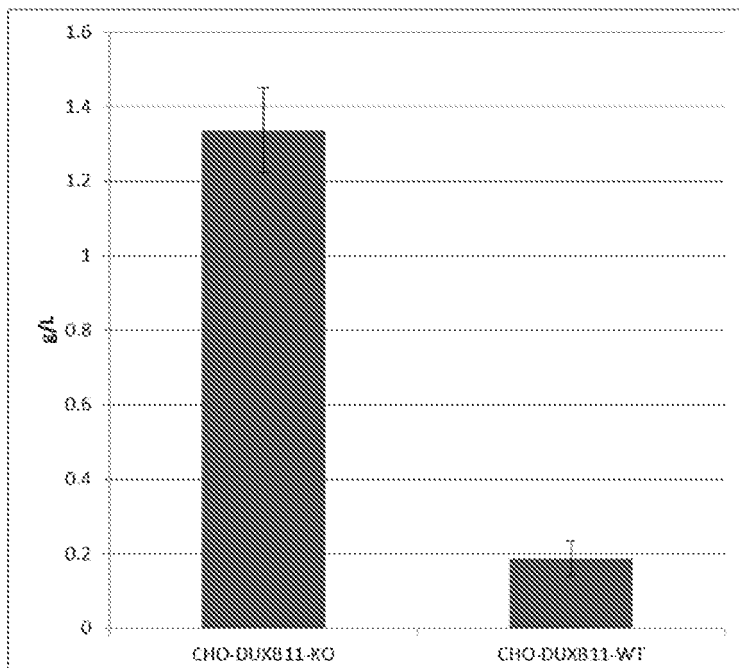


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Figure 16



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Figure 17:

Generic name	amino acid at position 42	yield mg/L	% Aggregation	% Full length protein after challenge (CHOK1 derived cells)	% Full length protein after challenge (CHO-DUXB11 derived cells)
hIGF1-Ea-del1-3, R37A, del71-72, R74Q, R77Q, R104Q-fc (SEQ ID No. 113) (batch 1)	glycine	11.33	18.3	21.6% $\pm$ 13.4% (n=4)	27.06%
hIGF1-Ea-hFc_mut02 (SEQ ID No.: 29)	serine	13.64	5.8	9.6% $\pm$ 13.9% (n=3)	N/A
hIGF1-Ea-hFc_mut03 (SEQ ID No.: 114)	glycine	5.17	41.8	44.9% $\pm$ 12.1% (n=3)	N/A
hIGF1-Ea-hFc_mut04 (SEQ ID No.: 30)	glycine	10.34	21.1	60.2% $\pm$ 12.2% (n=4)	73.6 %
hIGF1-Ea-hFc_mut12 (SEQ ID No.: 116)	glycine	8.46	17.7	44.3% $\pm$ 15.1% (n=3)	67.9%
hIGF1-Ea-hFc_mut13 (SEQ ID No.: 31)	glycine	10.18	24.0	64.2% $\pm$ 11.5% (n=4)	80.0%
hlgF1-Ea-Fc_mut 13/2_E (SEQ ID No.: 08)	serine	11.62	7.4	95.4% $\pm$ 4.4% (n=3)	97.3% $\pm$ 3.4% (n=2)
hlgF1-Ea-Fc_mut 13/2_A (SEQ ID No.: 09)	serine	11.72	8.6	93.4%	95.3%
hlgF1-Ea-Fc_mut 04/2_E (SEQ ID No.: 12)	serine	11.51	7.3	95.0% $\pm$ 4.6% (n=3)	98.7% $\pm$ 1.9% (n=2)

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Figure 18:

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ligand	Mean max fold increase vs control $\pm$ SD
Insulin	21.0 $\pm$ 6.9
IGF-1	3.7 $\pm$ 0.8
IGF-1 G42S	3.3 $\pm$ 1.1
hIGF1-Ea-mut 03	5.6 $\pm$ 2.0
hIGF1-Ea-mut 03-G42S	2.2 $\pm$ 0.5*

* $P < 0.05$ versus BVS855

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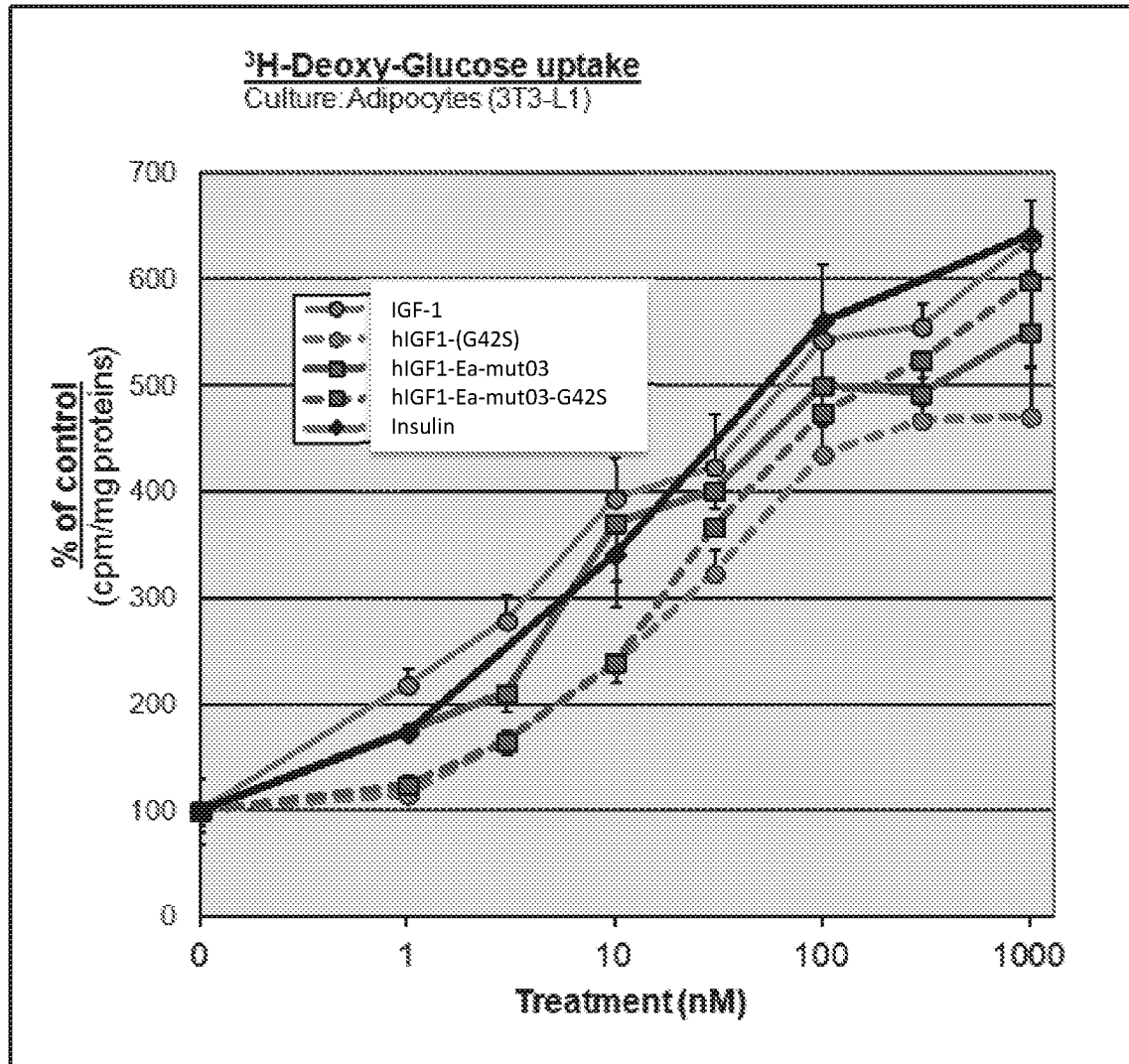
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5 Figure 19:

A



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5 Figure 19 continued:

B

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