1979_B06 MGVSDVPRDLEVVAATPTSLLISWSLPHAGHVNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSQMIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 80)
2062_G02 MGVSDVPRDLEVVAATPTSLLISWVSPRGRAR-YYRITYGETGGNSPVQEFTV*PGRGST*ATISGLKPGVDYTITVYAVTIYRDGMSHHDPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 81)
2522_C09 MGVSDVPRDLEVVAATPTSLLISWEVPRGLAR-YYRITYGETGGNSPVQEFTV*LGRGST*ATISGLKPGVDYTITVYAVTVYRDGPLLLAPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 82)
2523_G06 MGVSDVPRDLEVVAATPTSLLISWWAPLGLAR-YYRITYGETGGNSPVQEFTV*PGRGST*ATISGLKPGVDYTITVYAVTIFRTGMVQYDPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 83)
2524_C11 MGVSDVPRDLEVVAATPTSLLISWTLPHAGLAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTNSEIILYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 84)
2524_D09 MGVSDVPRDLEVVAATPTSLLISWYLPYPAHMNYYRITYGETGGNSPVQEFTV*PGRGLT*ATISGLKPGVDYTITVYAVTLTKSQILHHRPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 85)
2524_E10 MGVSDVPRDLEVVAATPTSLLISWSLPFAGHLNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 86)
2524_H05 MGVSDVPRDLEVVAATPTSLLISWSLPYSGLANYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTHSNIIRYVPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 87)
2524_H11 MGVSDVPRDLEVVAATPTSLLISWSLPHAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVSSTKVIVYLPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 88)
2525_B01 MGVSDVPRDLEVVAATPTSLLISWTLPNFGLINYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTITKSTIIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 89)
2525_D02 MGVSDVPRDLEVVAATPTSLLISWTLPHAGRAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTTTSVILYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 90)
2525_D05 MGVSDVPRDLEVVAATPTSLLISWSLPYAGHLNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSQLIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 91)
2525_F07 MGVSDVPRDLEVVAATPTSLLISWSLPYAAHMNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSQVIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 92)
2987_A06 MGVSDVPRDLEVVAATPTSLLISWSLPHAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 93)
2987_B04 MGVSDVPRDLEVVAATPTSLLISWSLPYPGHLNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 94)
2987_B09 MGVSDVPRDLEVVAATPTSLLISWTLPHAGRAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 95)
2987_C02 MGVSDVPRDLEVVAATPTSLLISWSLPYAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 96)
2987_D05 MGVSDVPRDLEVVAATPTSLLISWSLPHAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 97)
2987_E03 MGVSDVPRDLEVVAATPTSLLISWSLPYPGHLNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 98)
2987_E08 MGVSDVPRDLEVVAATPTSLLISWTLPHAGRAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVSSTKVIHYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 99)
2987_F01 MGVSDVPRDLEVVAATPTSLLISWSLPYAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTRSKIIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 100)
2987_F06 MGVSDVPRDLEVVAATPTSLLISWSLPHAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKVIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 101)
2987_G04 MGVSDVPRDLEVVAATPTSLLISWSLPYPGHLNYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKVIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 102)
2987_G09 MGVSDVPRDLEVVAATPTSLLISWTLPHAGRAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVSSTKVIVYLPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 103)
2987_H02 MGVSDVPRDLEVVAATPTSLLISWSLPYAGHAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTKSKVIHYMPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 104)
2987_H07 MGVSDVPRDLEVVAATPTSLLISWTLPHAGRAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTTTKVIHYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 105)
3006_A10 MGVSDVPRDLEVVAATPTSLLISWDAPGGLAR-YYRITYGETGGNSPVQEFTV*IGRGST*ATISGLKPGVDYTITVYAVTIDRDGVNHFAPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 106)
3007_B08 MGVSDVPRDLEVVAATPTSLLISWSLPTPGLAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTHHGVIGYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 107)
3007_C09 MGVSDVPRDLEVVAATPTSLLISWSLPHRGVANYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTGANVIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 108)
3007_C10 MGVSDVPRDLEVVAATPTSLLISWSLPSSGVAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTNTGVIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 109)
3008_A03 MGVSDVPRDLEVVAATPTSLLISWSLPHHGFGHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTATGIIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 110)
3008_B08 MGVSDVPRDLEVVAATPTSLLISWSLPHAGDAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTRAGFYRYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 111)
3008_D04 MGVSDVPRDLEVVAATPTSLLISWSLPHNGVAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTREEVISYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 112)
3008_F01 MGVSDVPRDLEVVAATPTSLLISWSLPRQGLANYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTAAGVIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 113)
3008_G01 MGVSDVPRDLEVVAATPTSLLISWSLPGPGHFHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTANQPIIYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 114)
3008_G03 MGVSDVPRDLEVVAATPTSLLISWSLPHPGLGHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTITPETIIVYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 115)
3115_D04 MGVSDVPRDLEVVAATPTSLLISWDAPRGLAR-YYRITYGETGGNSPVQEFTV*FGRGTT*ATISGLKPGVDYTITVYAVTIDRDGTRSFDPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 116)
3115_E06 MGVSDVPRDLEVVAATPTSLLISWDAPAGLAR-YYRITYGETGGNSPVQEFTV*VGRGNT*ATISGLKPGVDYTITVYAVTIFRDGPVTWDPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 117)
3116_A06 MGVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 118)
3116_A07 MGVSDVPRDLEVVAATPTSLLISWDAPKGLAR-YYRITYGETGGNSPVQEFTV*VGRGNT*ATISGLKPGVDYTITVYAVTIFRDGPVTWDPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 119)
3116_C01 MGVSDVPRDLEVVAATPTSLLISWSLPNPGIAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTLTGSDTIFYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 120)
3116_C06 MGVSDVPRDLEVVAATPTSLLISWSLPRPGNAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTGKDVIKYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 121)
3116_H06 MGVSDVPRDLEVVAATPTSLLISWDAPAGLAR-YYRITYGETGGNSPVQEFTV*VGRGNT*ATISGLKPGVDYTITVYAVTIFRDGVVNYGPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 122)
3146_A08 MGVSDVPRDLEVVAATPTSLLISWSLPNPGNAHYYRITYGETGGNSPVQEFTV*PGRGVTA*TISGLKPGVDYTITVYAVTVTDTGFITYKPISINYRTEIDKPSQHHHHHH (SEQ ID NO: 123)

Fig. 1

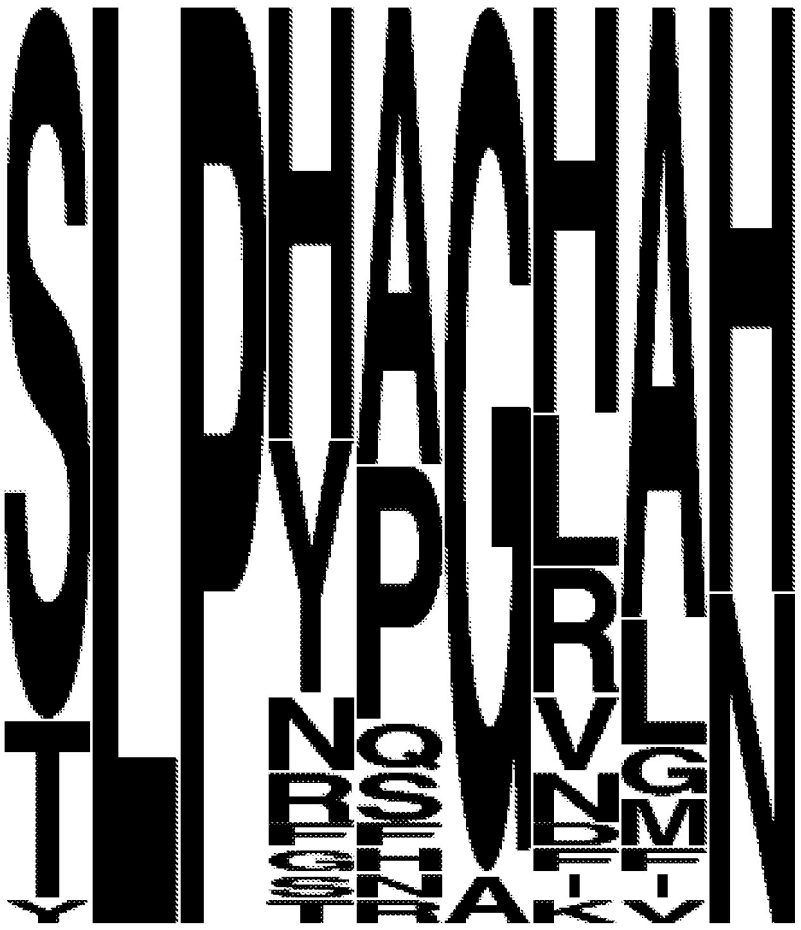


Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6

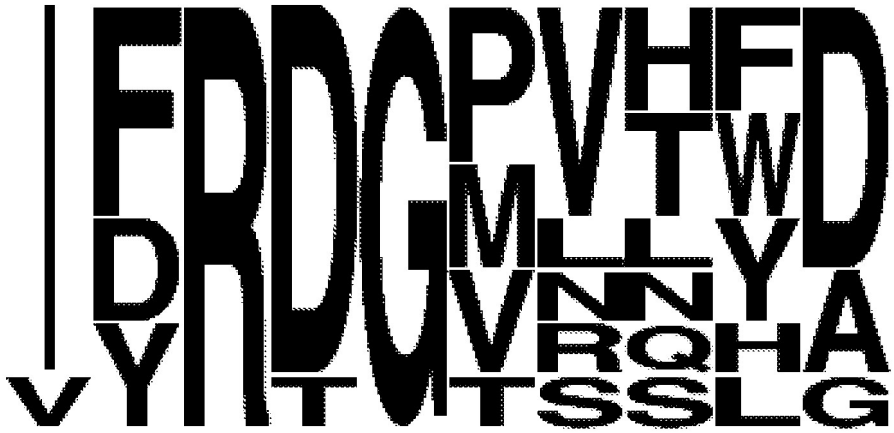


Fig. 7

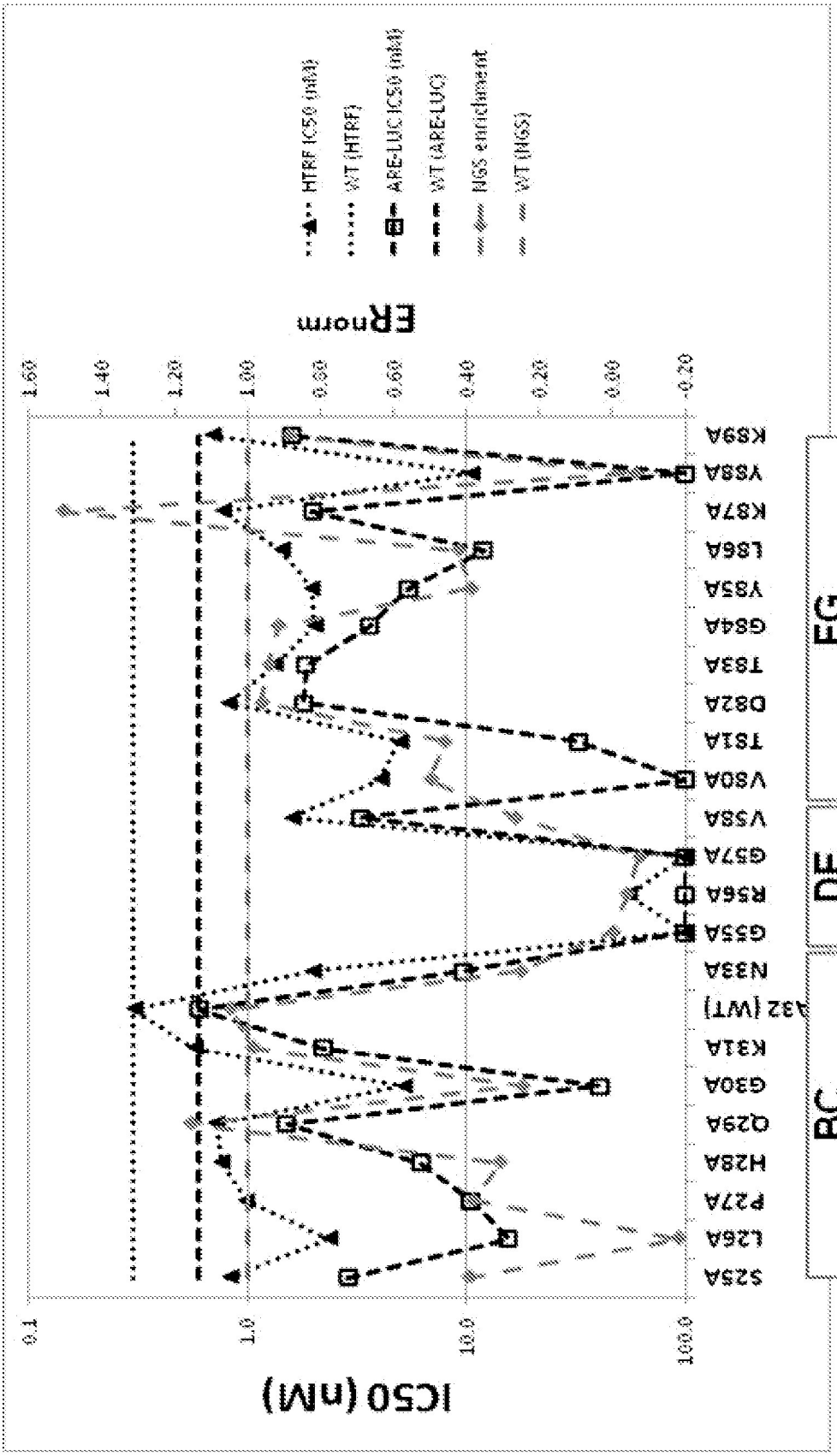

HTRF IC50 (nM)
WT (HTRF)
ARE-LUC IC50 (nM)
WT (ARE-LUC)
NGS enrichment
WT (NGS)
ERnorm
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
IC50 (nM)
0.1
1.0
10.0
100.0
S25A
L26A
P27A
H28A
Q29A
G30A
K31A
N32 (WT)
N33A
G55A
R56A
G57A
V58A
T81A
D82A
T83A
G84A
Y85A
L86A
K87A
Y88A
K89A
BC
DE
FG

Fig. 8

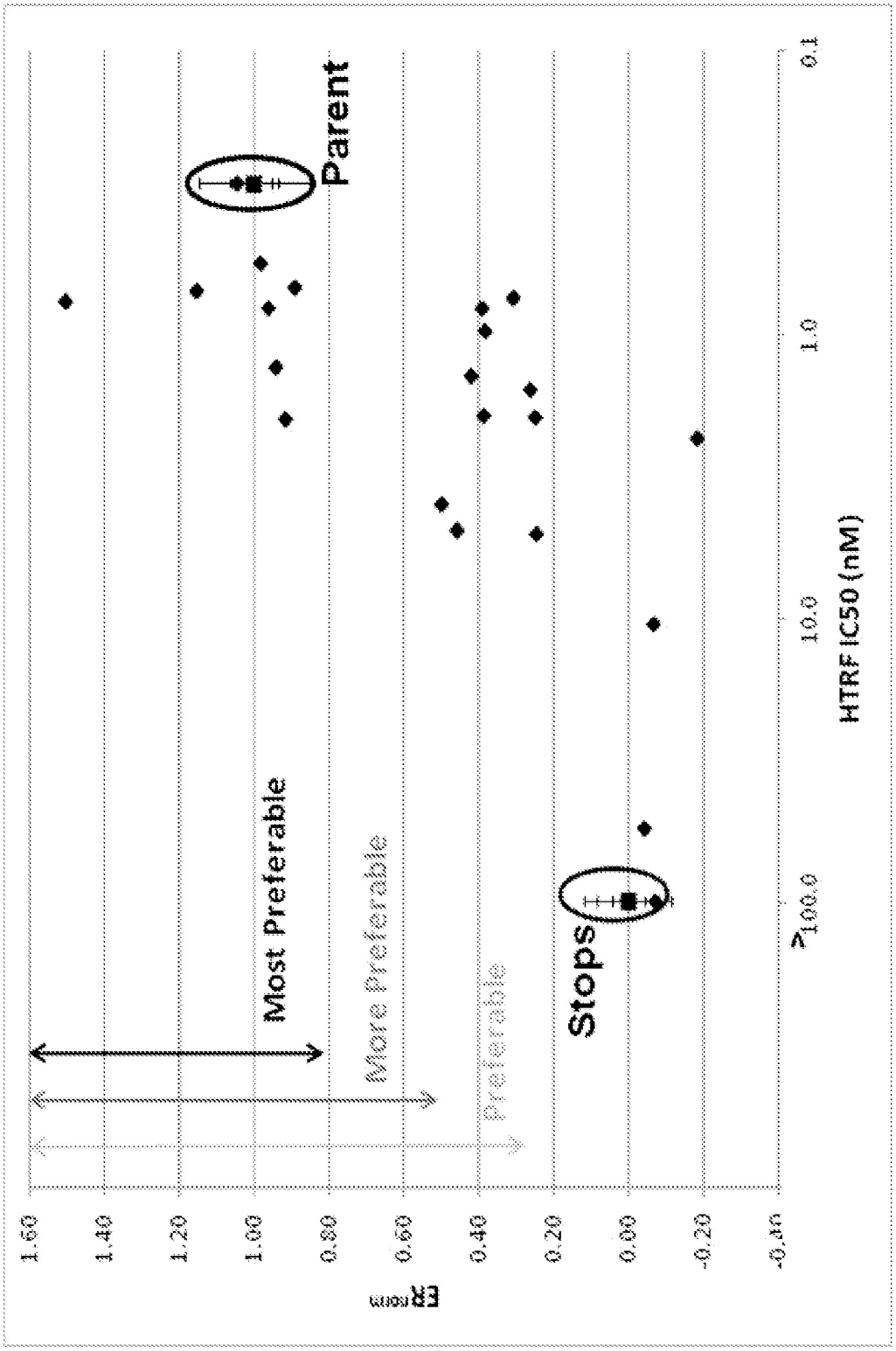

Parent
Most Preferable
More Preferable
Preferable
Stops
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
>100.0
10.0
1.0
0.1
HTRF IC50 (nM)

Fig. 9

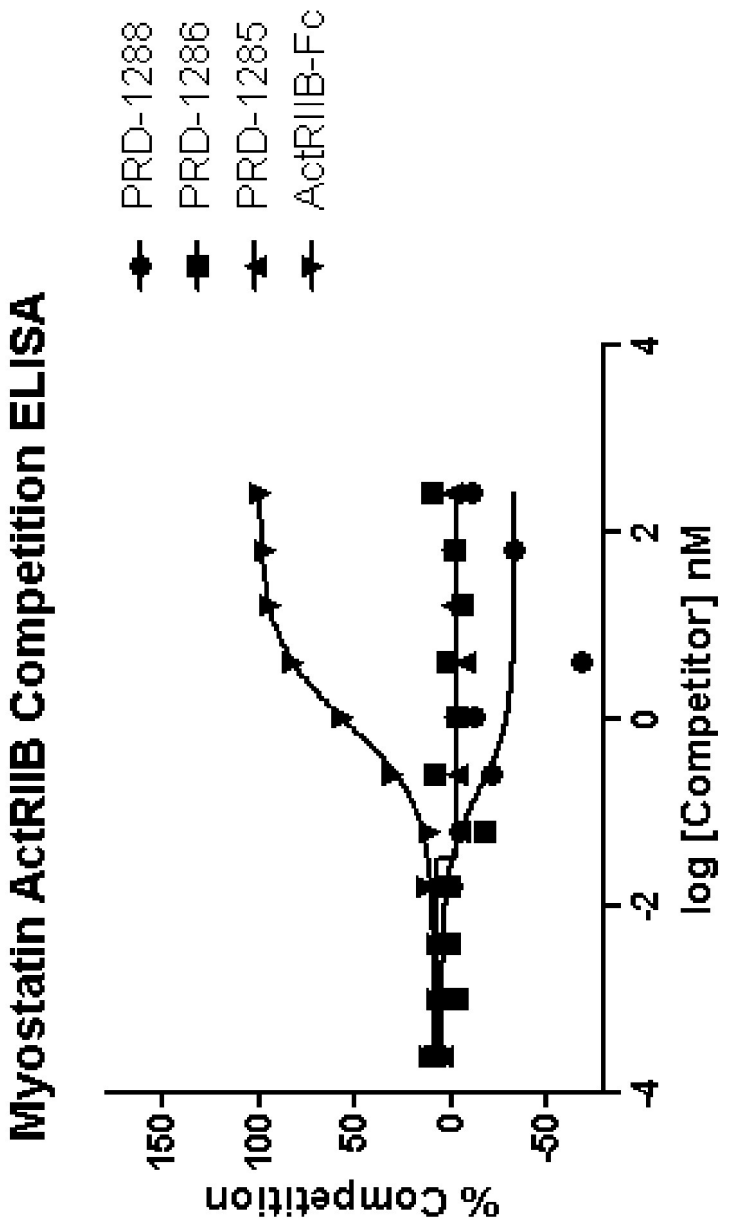

Myostatin ActRIIB Competition ELISA
PRD-1288
PRD-1286
PRD-1285
ActRIIB-Fc
% Competition
150
100
50
0
-50
-4
-2
0
2
4
log [Competitor] nM

Fig. 10

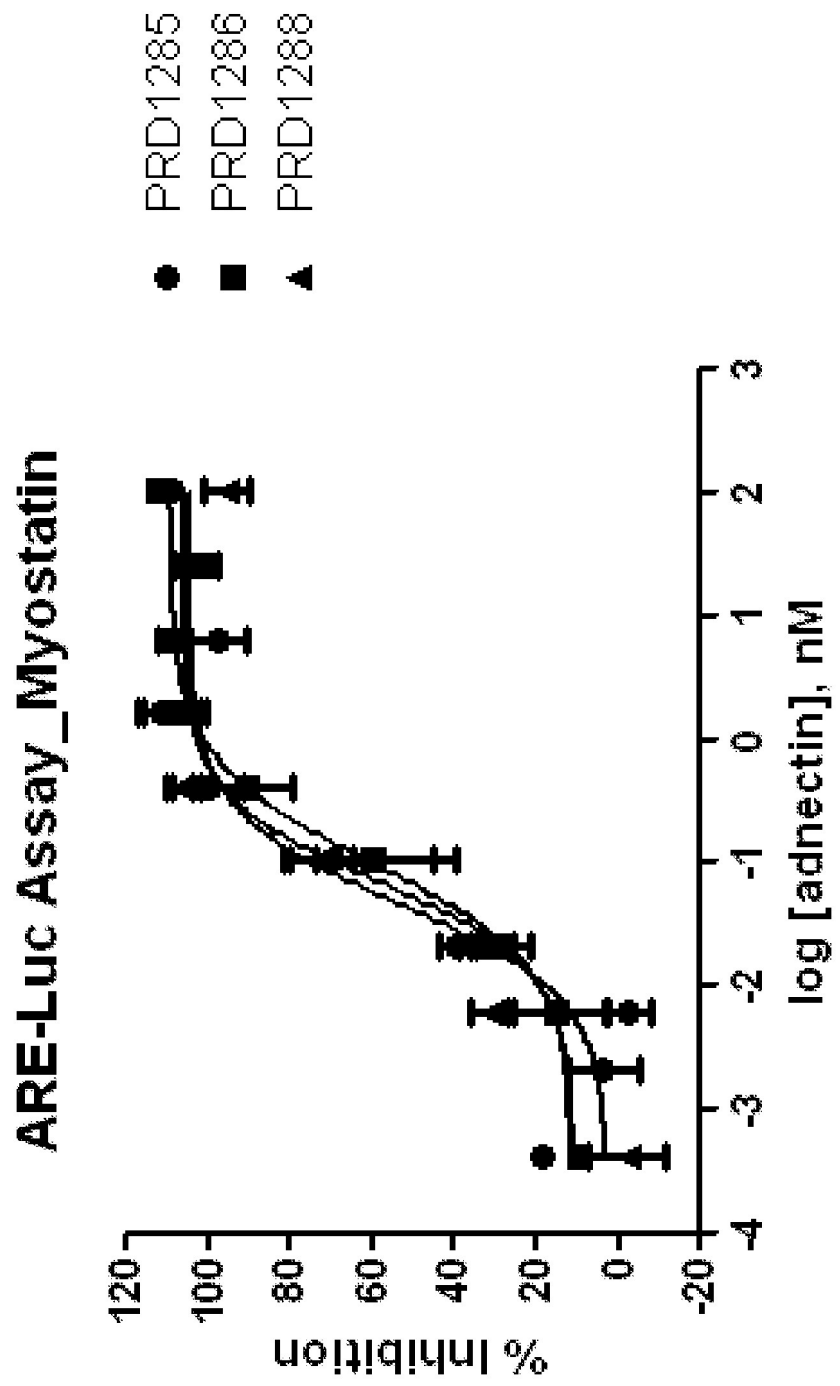
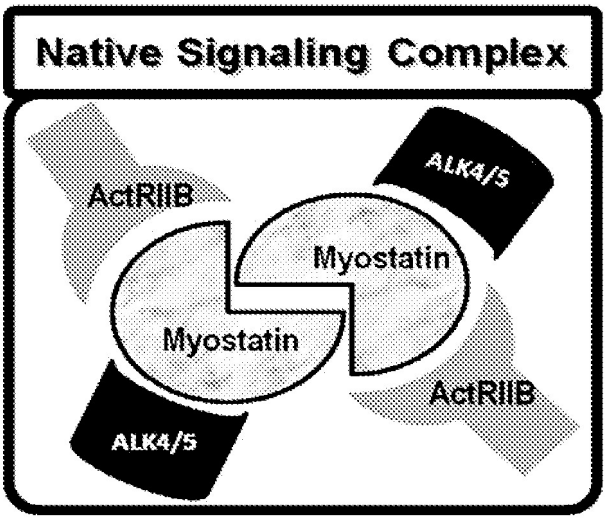

ARE-Luc Assay_Myostatin
PRD1285
PRD1286
PRD1288
% Inhibition
120
100
80
60
40
20
0
-20
-4
-3
-2
-1
0
1
2
3
log [adnectin], nM

Fig. 11

A.


Native Signaling Complex
ActRIIB
ALK4/5
Myostatin
Myostatin
ActRIIB
ALK4/5

B.

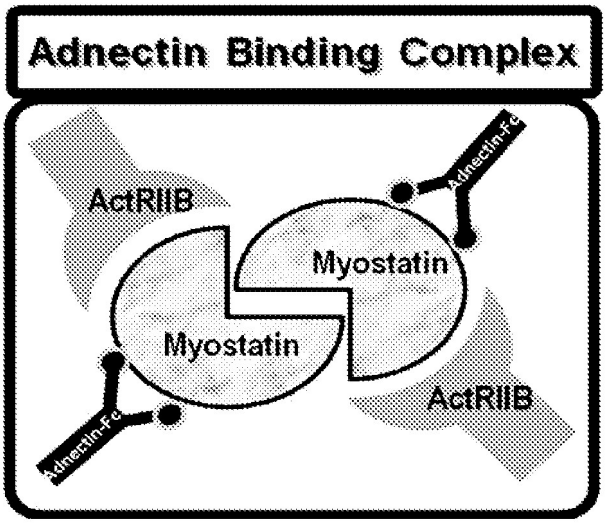

Adnectin Binding Complex
ActRIIB
Adnectin-Fc
Myostatin
Myostatin
ActRIIB
Adnectin-Fc

Fig. 12


HDX-MS
Region 2
HDX-MS
Region 1
Site 2
ActRIIB
binding site
Site 1
ALK4
binding site
Site 2
ActRIIB
binding site
HDX-MS
Region 2
HDX-MS
Region 1
Site 1
ALK4
binding site

Fig. 13A



Fig. 13B

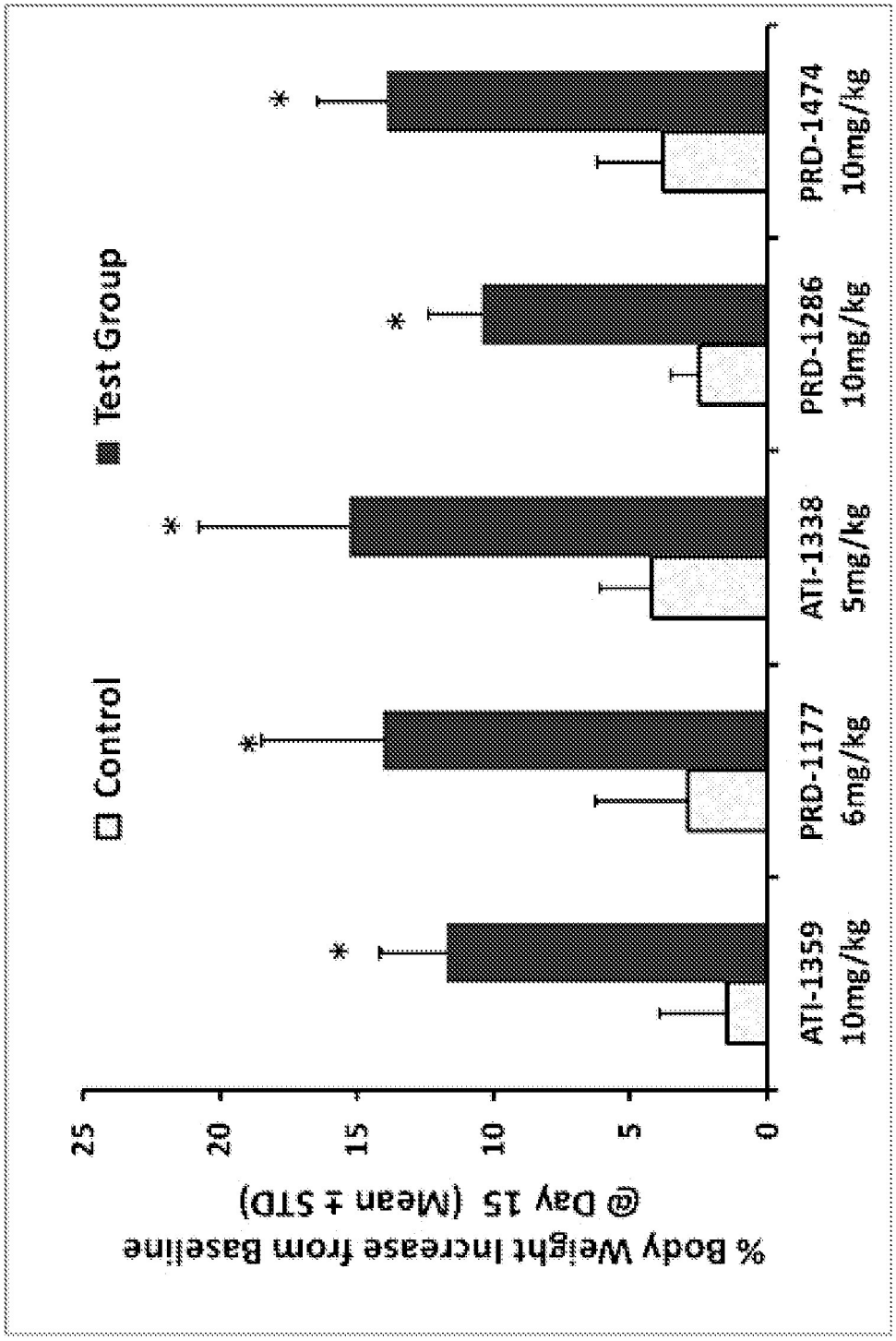

% Body Weight Increase from Baseline
@ Day 15 (Mean ± STD)
25
20
15
10
5
0
Control
Test Group
*
*
*
*
ATI-1359
10mg/kg
PRD-1177
6mg/kg
ATI-1338
5mg/kg
PRD-1286
10mg/kg
PRD-1474
10mg/kg

Fig. 14

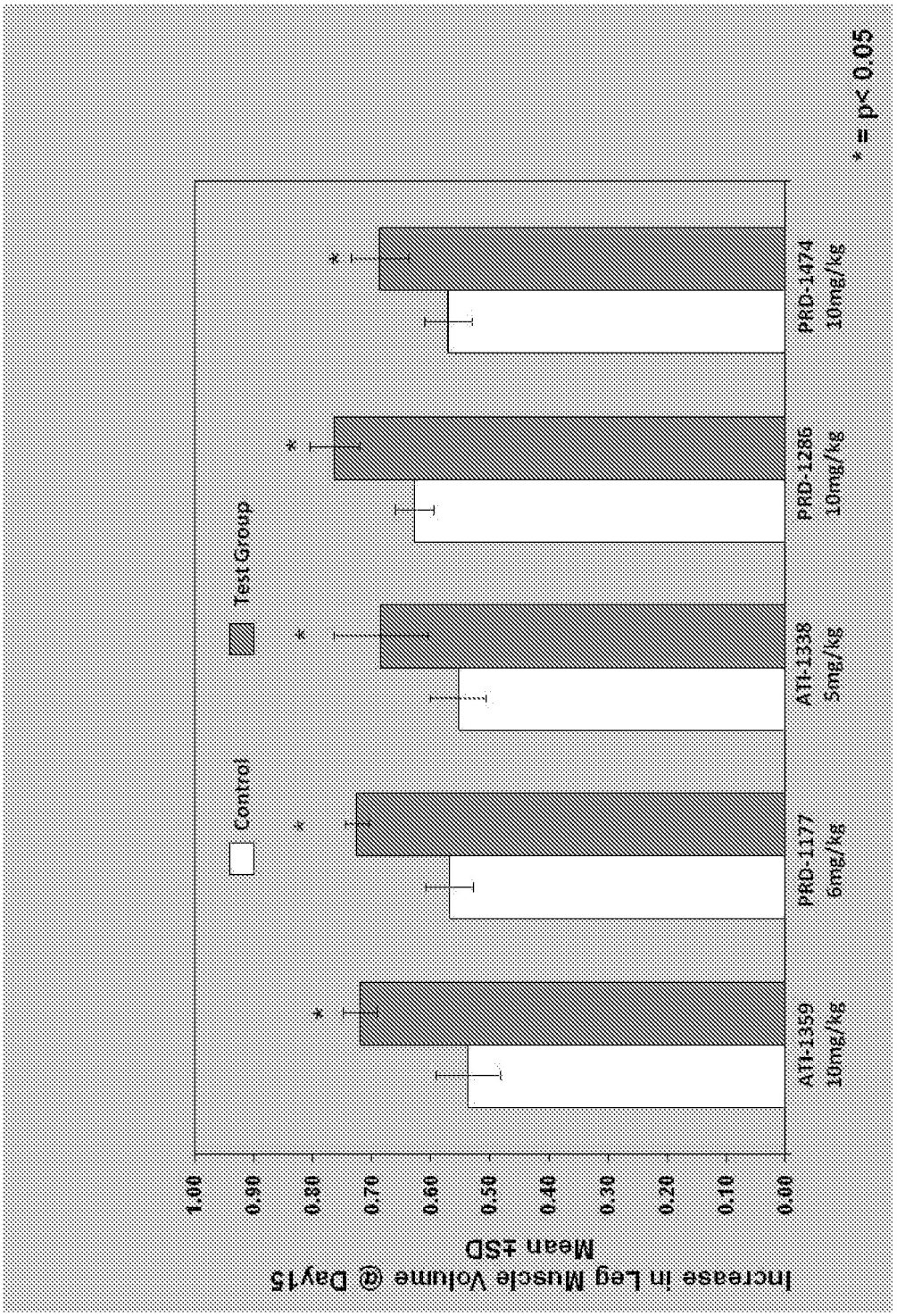

Increase in Leg Muscle Volume @ Day15
Mean ±SD
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Control
Test Group
*
A11-1359 10mg/kg
PRD-1177 6mg/kg
A11-1338 5mg/kg
PRD-1286 10mg/kg
PRD-1474 10mg/kg
* = p< 0.05

Fig. 15

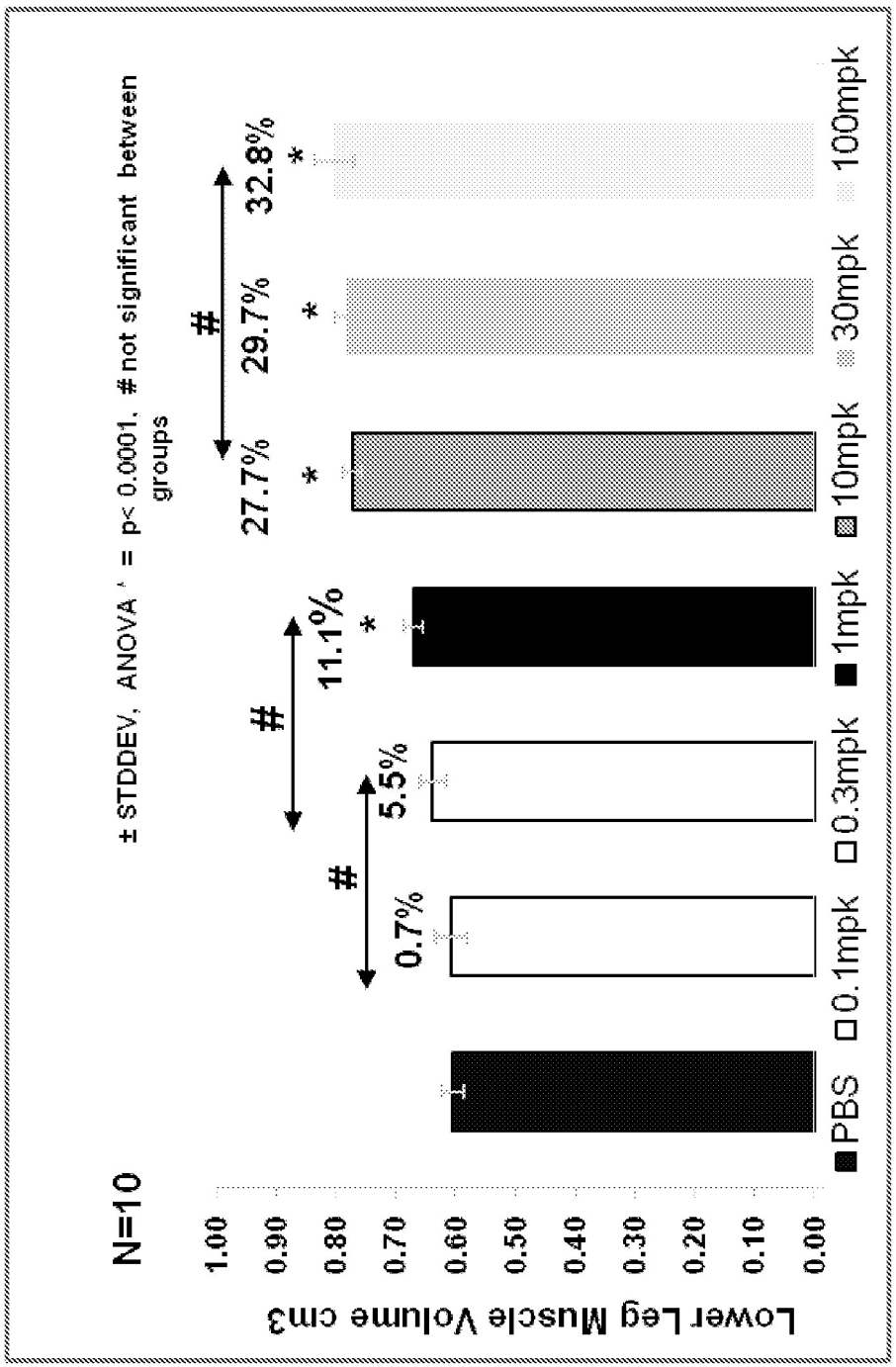

N=10
± STDDEV, ANOVA ' = p< 0.0001, # not significant between groups
Lower Leg Muscle Volume cm3
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
PBS
0.1mpk
0.3mpk
1mpk
10mpk
30mpk
100mpk
#
0.7%
#
5.5%
#
11.1%
*
27.7%
*
#
29.7%
*
32.8%
*

Fig. 16