Figure 1

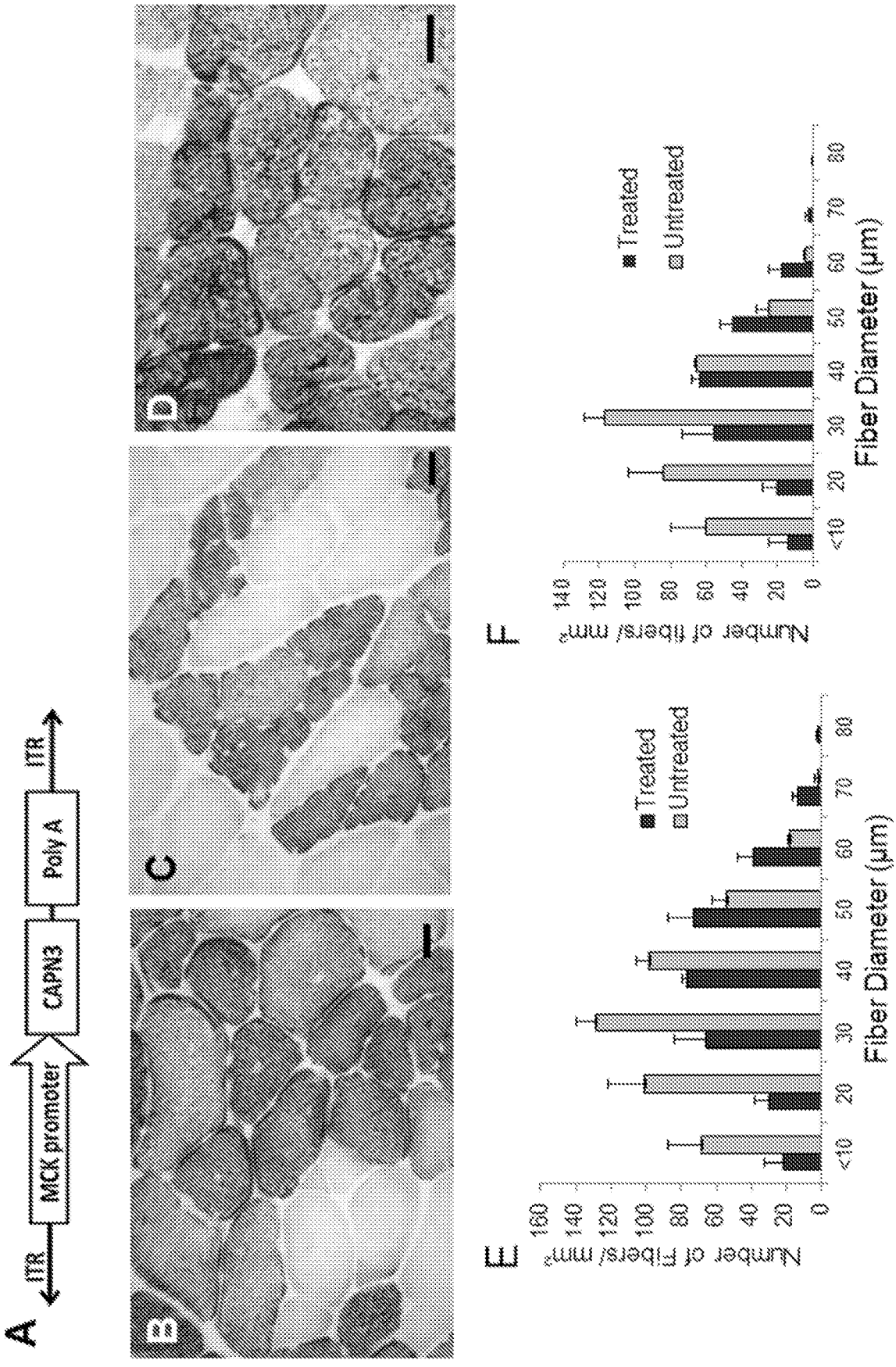

A
ITR
MCK promoter
CAPN3
Poly A
ITR
B
C
D
E
Number of fibers/ mm²
Treated
Untreated
Fiber Diameter (µm)
F
Number of fibers/ mm²
Treated
Untreated
Fiber Diameter (µm)

Figure 2

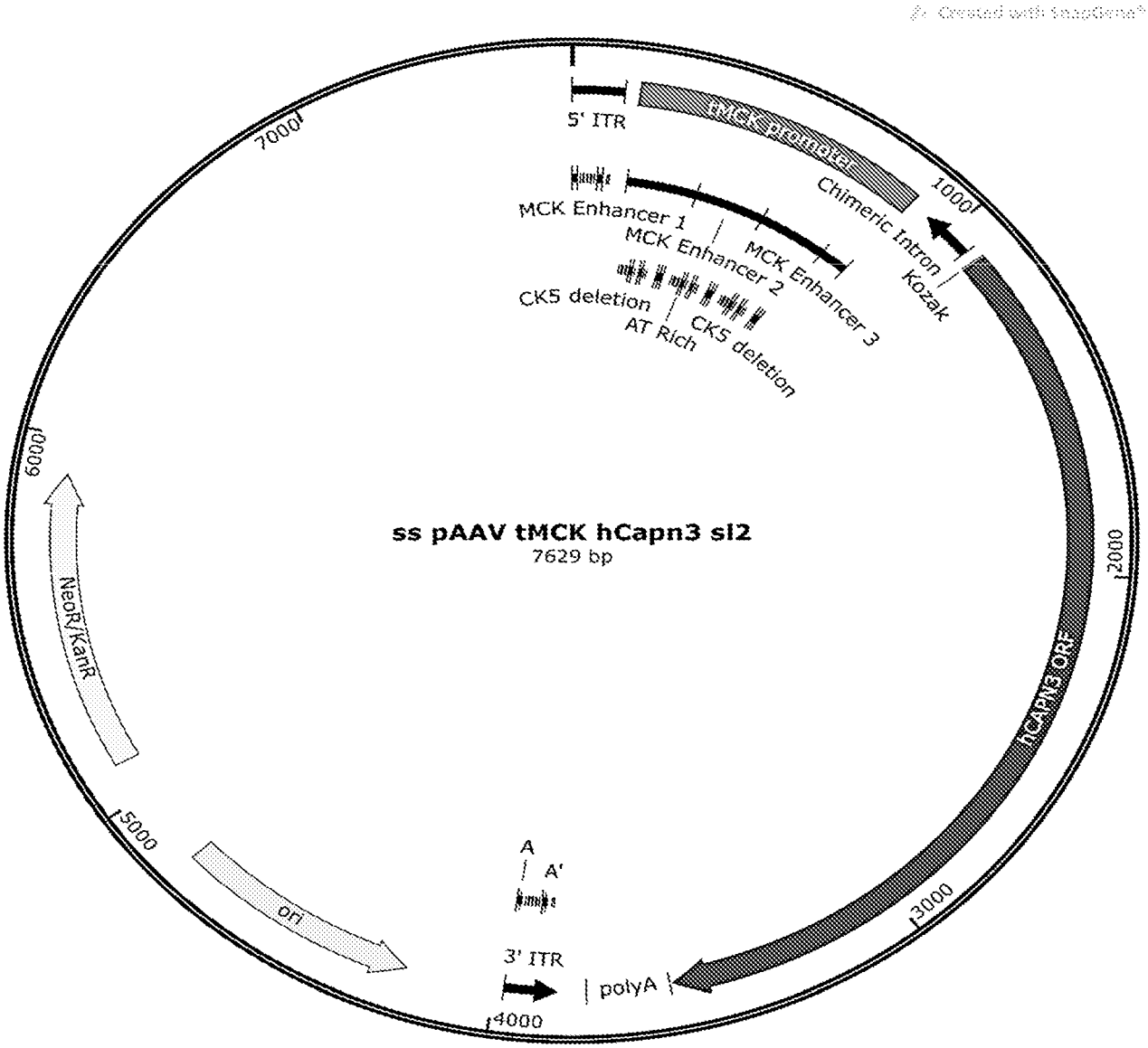
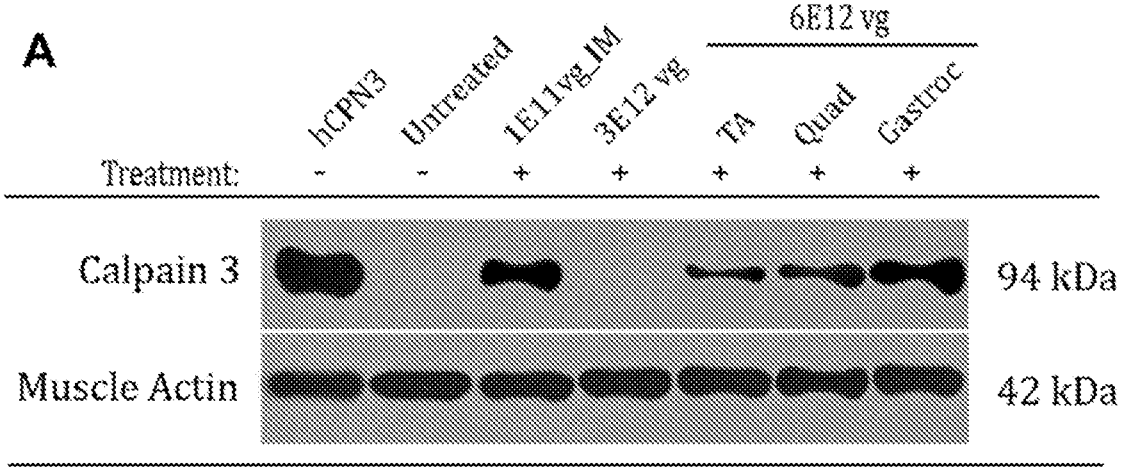
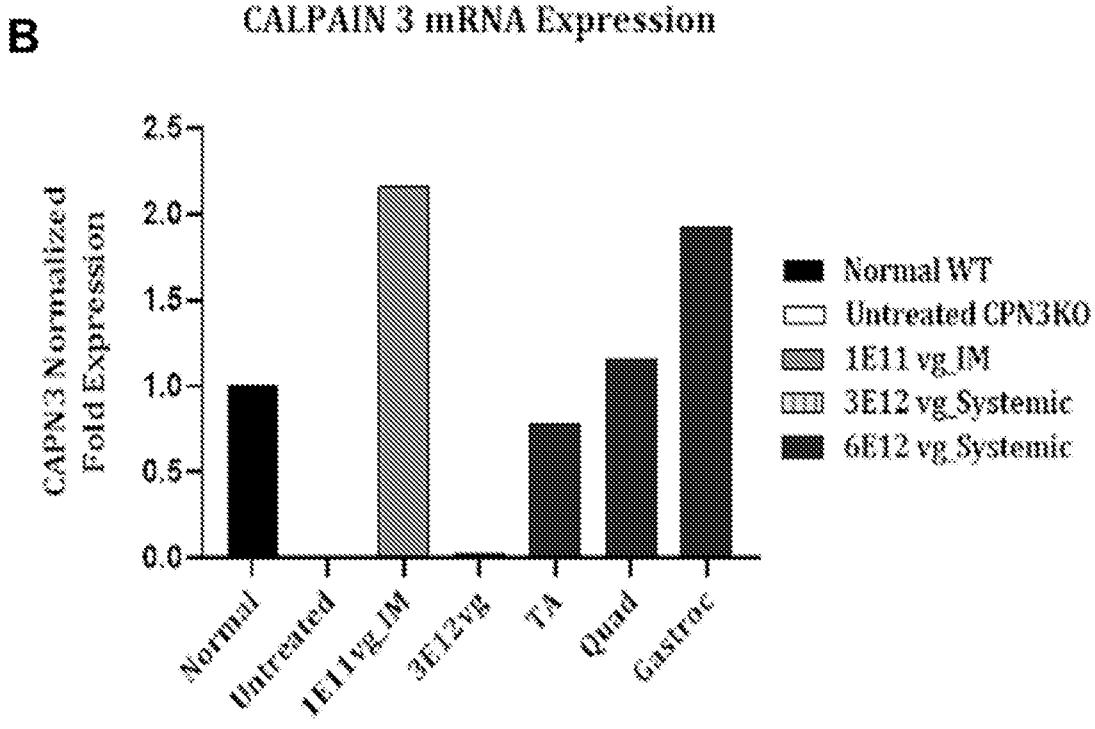

Created with SnapGene®
5' ITR
MCK promoter
Chimeric Intron
Kozak
MCK Enhancer 1
MCK Enhancer 2
MCK Enhancer 3
CK5 deletion
AT Rich
CK5 deletion
ss pAAV tMCK hCapn3 sI2
7629 bp
hCAPN3 ORF
NeoR/KanR
ori
A
A'
3' ITR
polyA
1000
2000
3000
4000
5000
6000
7000

Figure 3


A
6E12 vg
hCPN3
Untreated
1E11vg_IM
3E12 vg
TA
Quad
Gastroc
Treatment:
- - + + + + +
Calpain 3
94 kDa
Muscle Actin
42 kDa


B
CALPAIN 3 mRNA Expression
CAPN3 Normalized Fold Expression
2.5
2.0
1.5
1.0
0.5
0.0
Normal
Untreated
1E11vg_IM
3E12vg
TA
Quad
Gastroc
Normal WT
Untreated CPN3KO
1E11 vg_IM
3E12 vg_Systemic
6E12 vg_Systemic

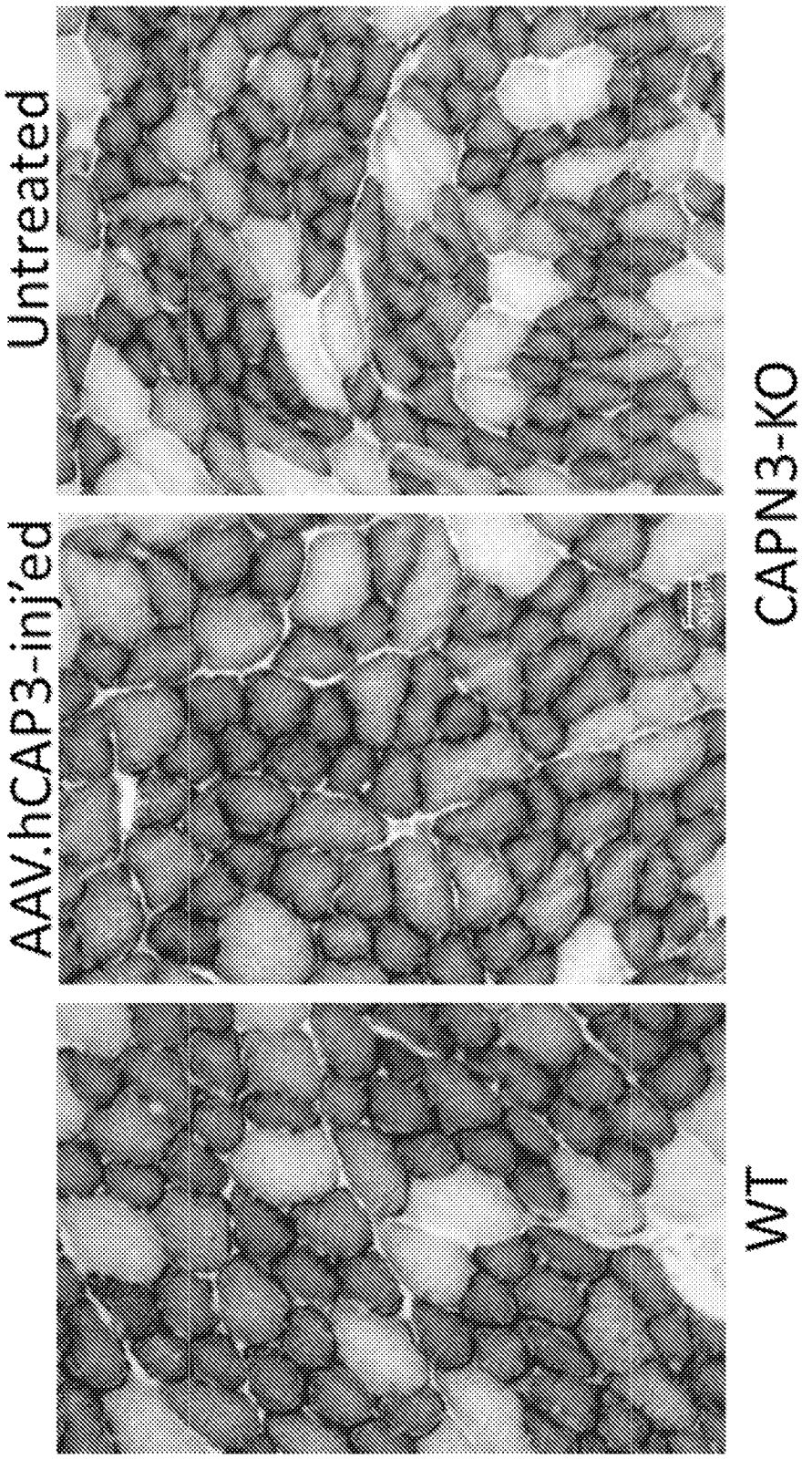


Figure 4

Figure 5

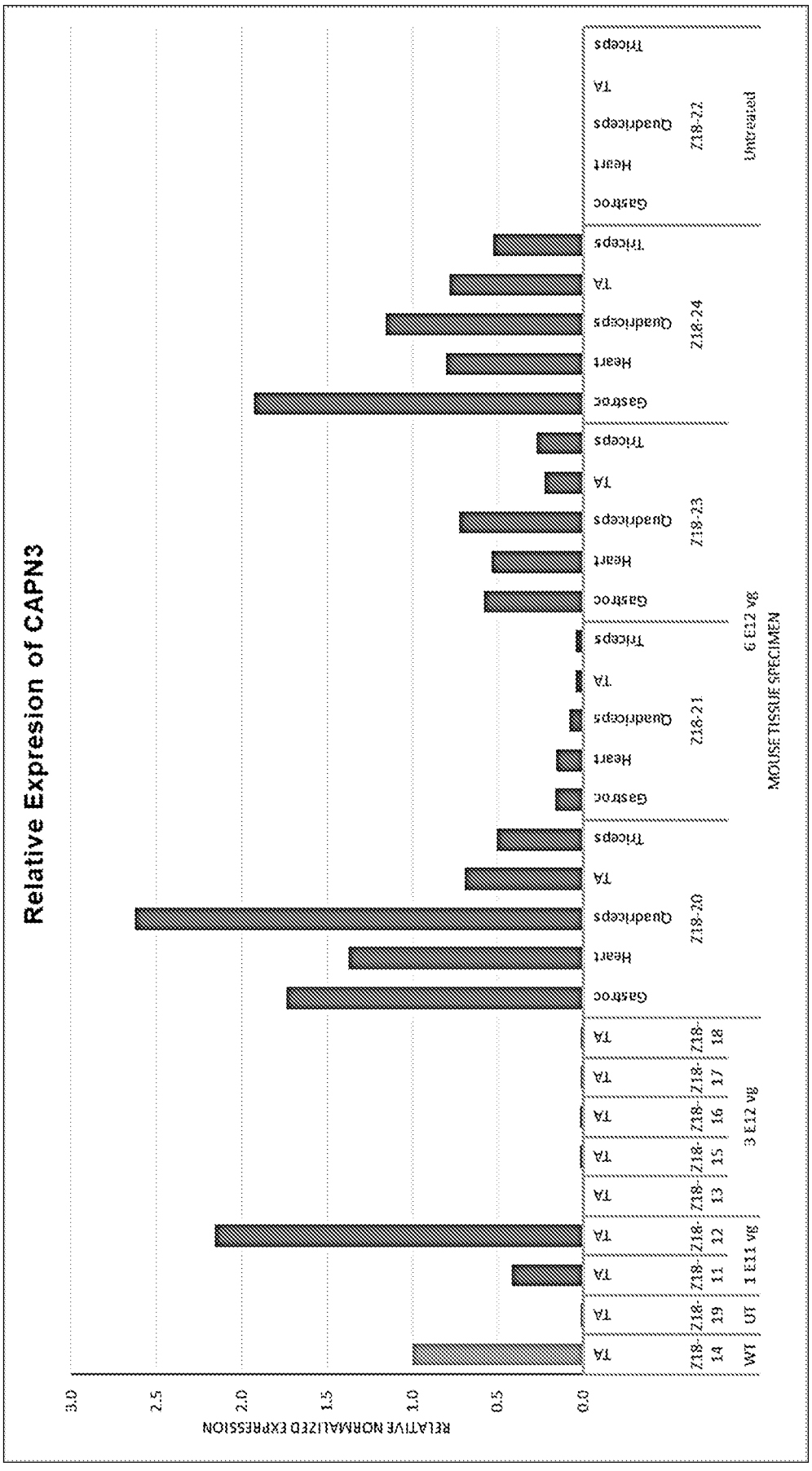

Relative Expresion of CAPN3
RELATIVE NORMALIZED EXPRESSION
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MOUSE TISSUE SPECIMEN
WT
Off
1 E11 vg
3 E12 vg
6 E12 vg
Untreated
Z18-14
Z18-19
Z18-11
Z18-12
Z18-13
Z18-15
Z18-16
Z18-17
Z18-18
Z18-20
Z18-21
Z18-23
Z18-24
Z18-22
TA
Gastroc
Heart
Quadriceps
Triceps

Figure 6

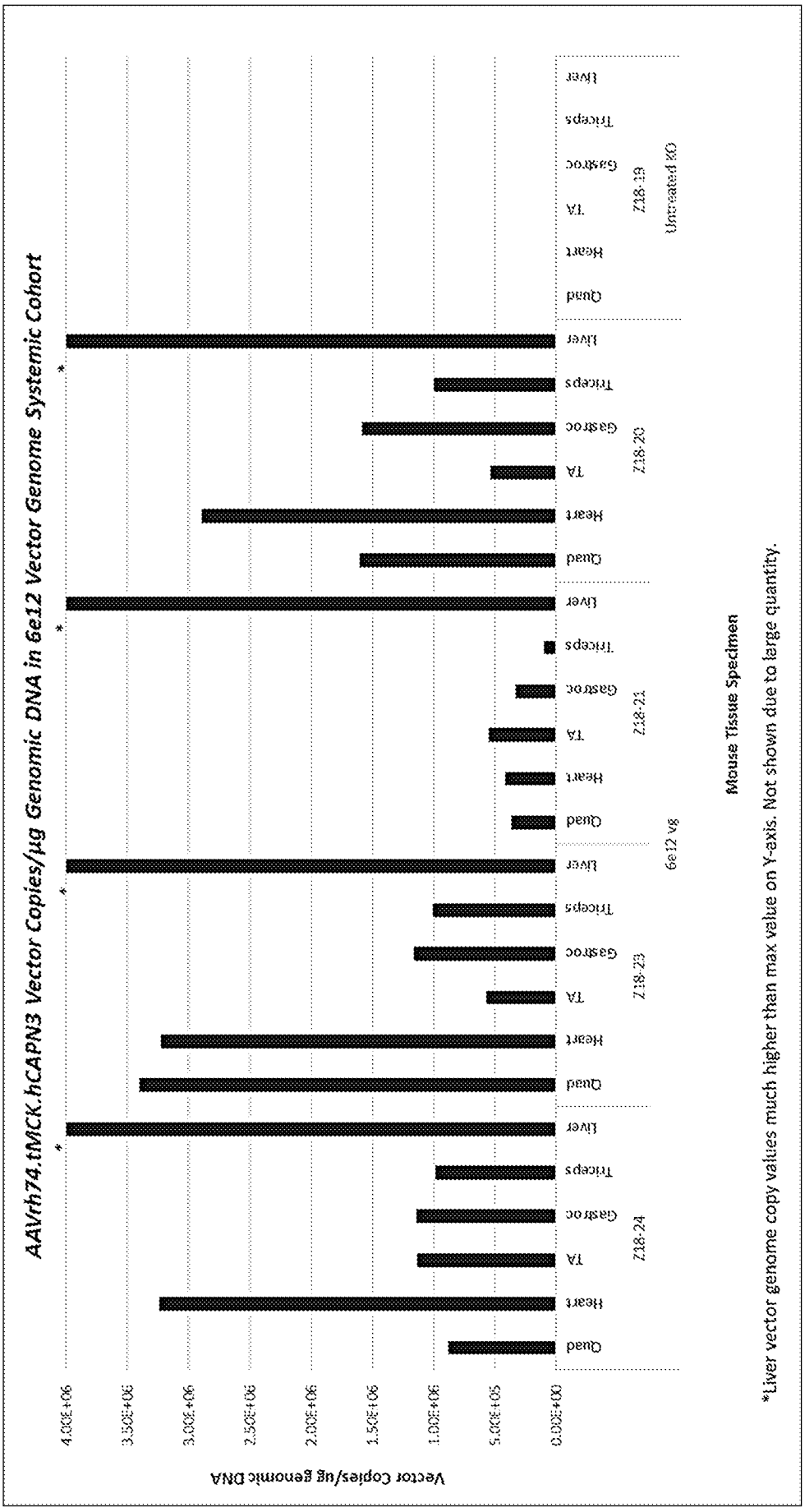

AAVrh74.tMCK.hCAPN3 Vector Copies/µg Genomic DNA in 6e12 Vector Genome Systemic Cohort
Vector Copies/ug genomic DNA
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Quad
Heart
TA
Gastroc
Triceps
Liver
Z18-24
Z18-23
Z18-21
Z18-20
Z18-19
Untreated KO
6e12 vg
*
Mouse Tissue Specimen
*Liver vector genome copy values much higher than max value on Y-axis. Not shown due to large quantity.

Figure 7

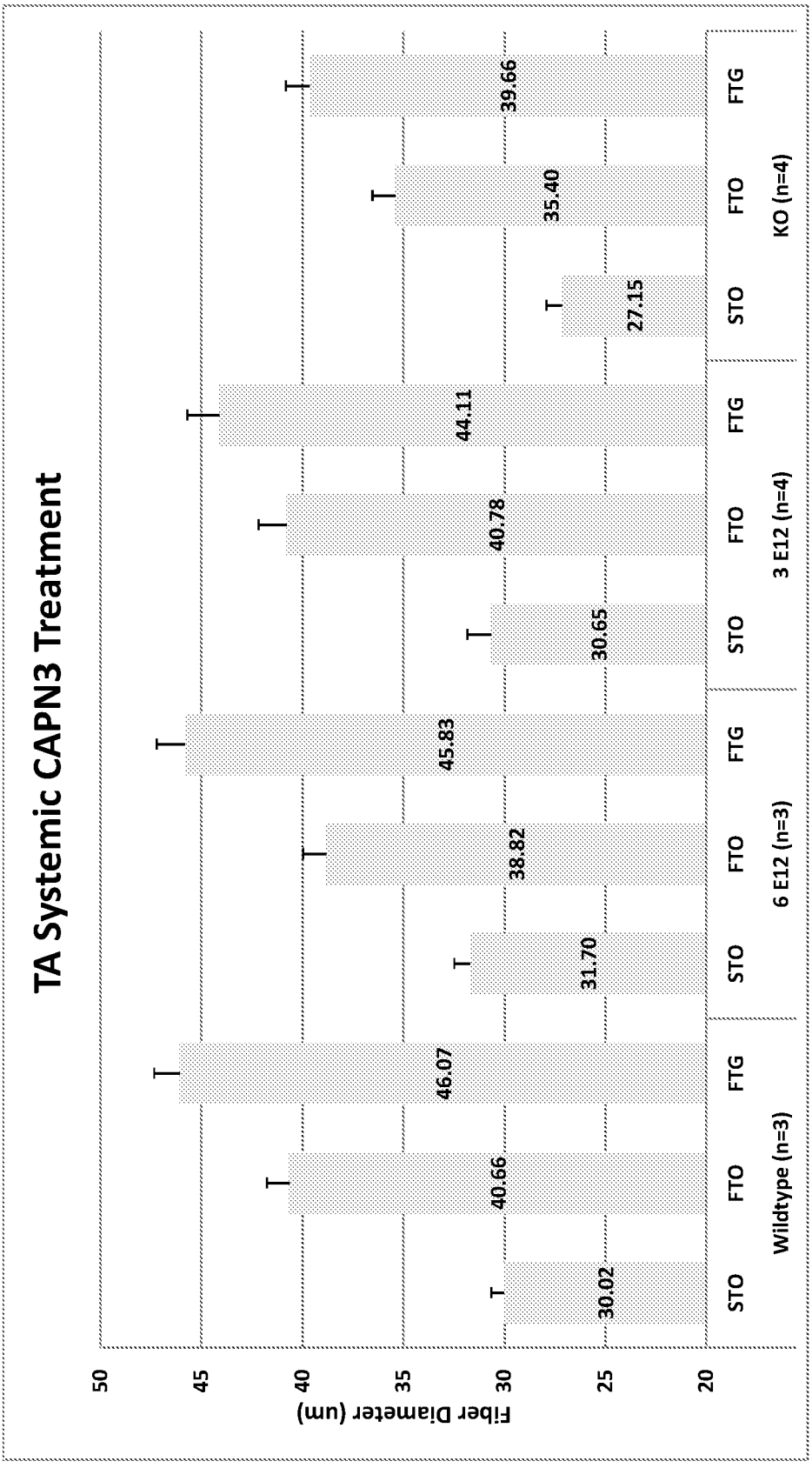
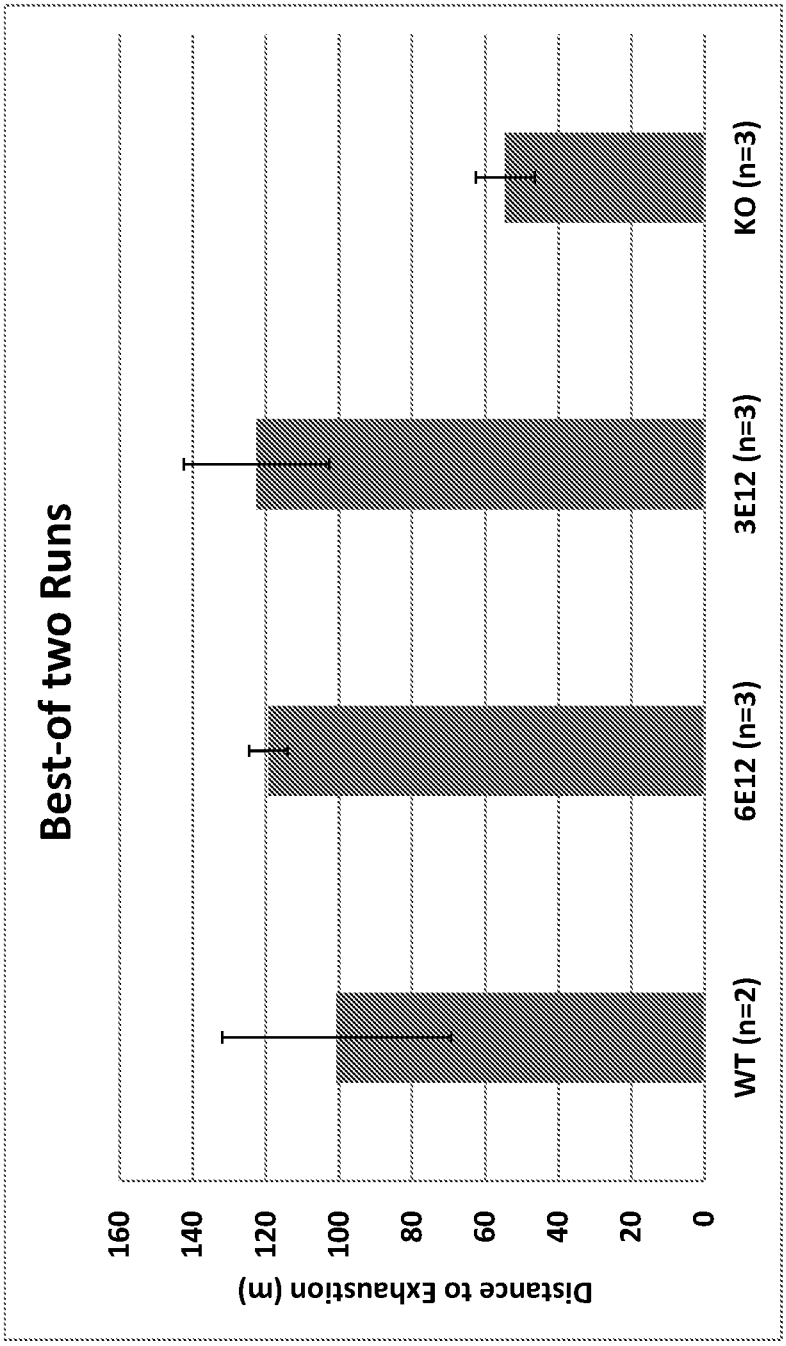

TA Systemic CAPN3 Treatment
Fiber Diameter (um)
50
45
40
35
30
25
20
STO
FTO
FTG
Wildtype (n=3)
30.02
40.66
46.07
6 E12 (n=3)
31.70
38.82
45.83
3 E12 (n=4)
30.65
40.78
44.11
KO (n=4)
27.15
35.40
39.66

Figure 8A


Best-of two Runs
Distance to Exhaustion (m)
0
20
40
60
80
100
120
140
160
WT (n=2)
6E12 (n=3)
3E12 (n=3)
KO (n=3)

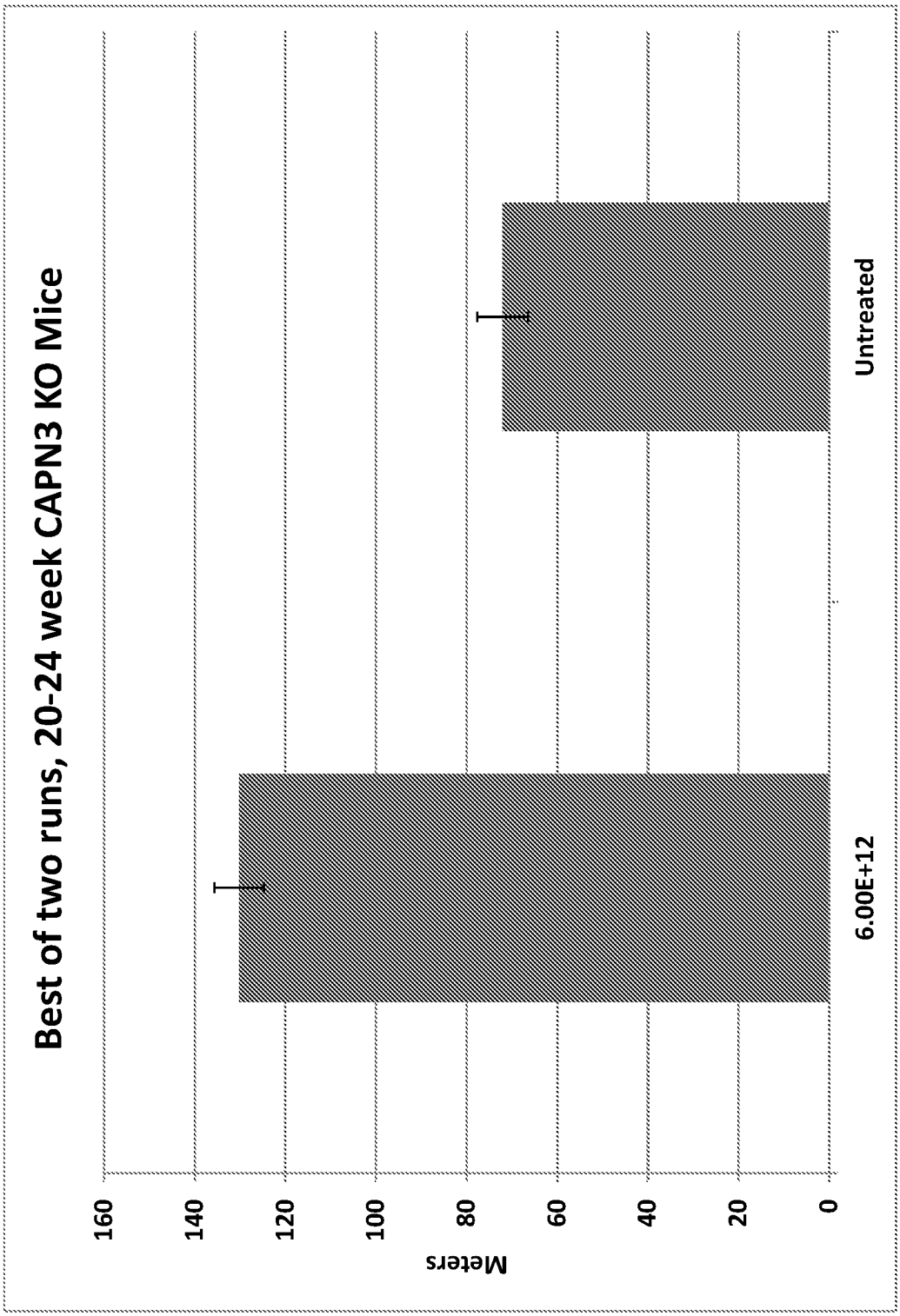

Best of two runs, 20-24 week CAPN3 KO Mice
Meters
160
140
120
100
80
60
40
20
0
6.00E+12
Untreated

Figure 8B

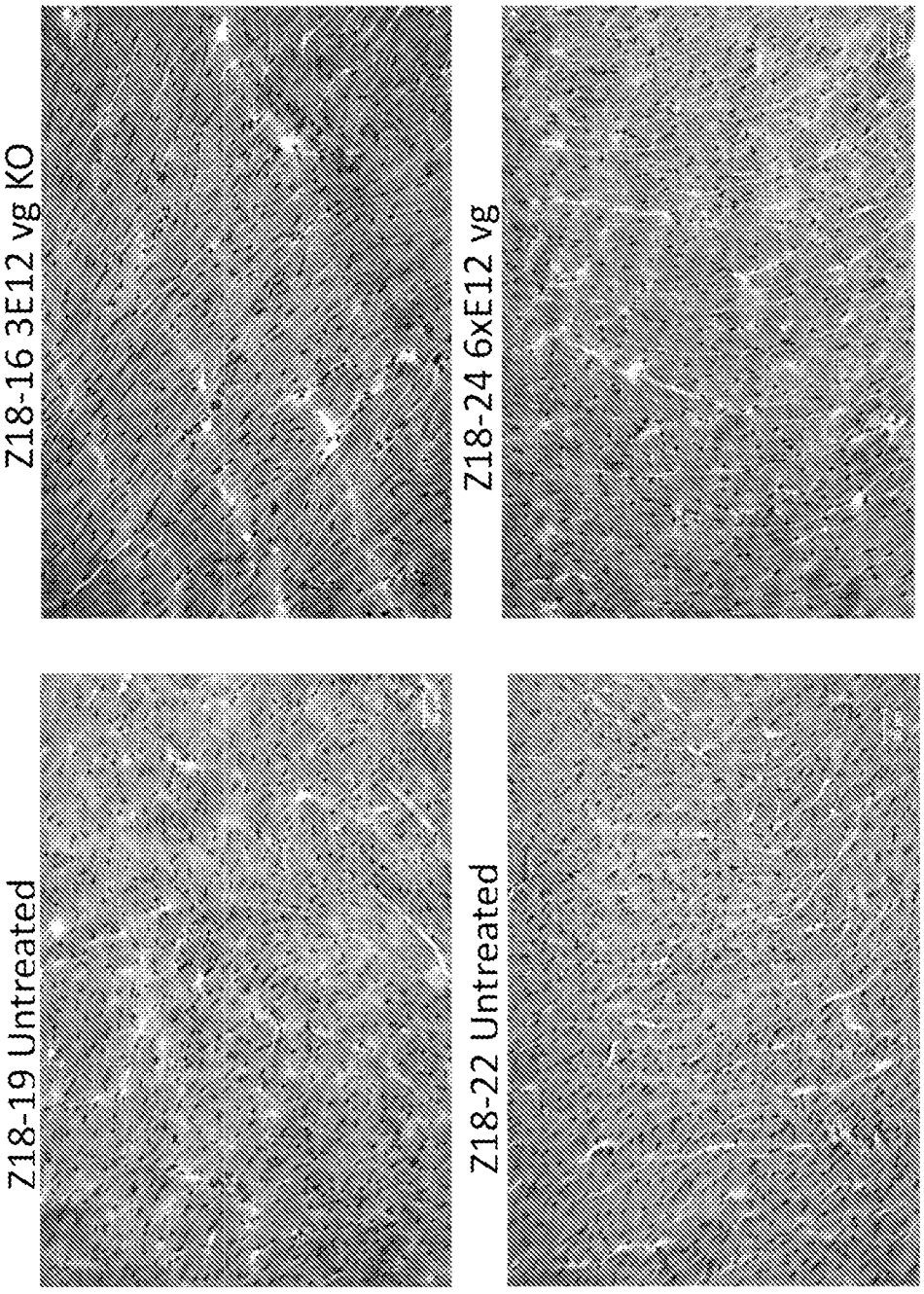
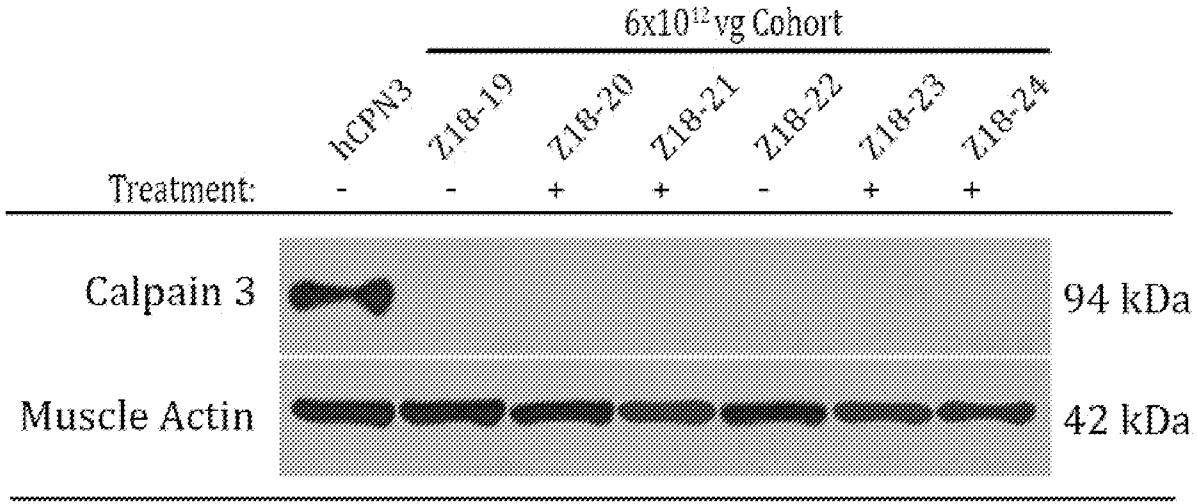

Z18-16 3E12 vg KO
Z18-24 6xE12 vg
Z18-19 Untreated
Z18-22 Untreated

Figure 9

Figure 10

Assessment of Cardiac Calpain 3 protein expression
Following systemic AAVrh.74 tMCK.CAPN3 Gene Delivery



Full-length Calpain 3 protein is below the limit of detection.
6×10^{12} vg AAVrh74.tMCK.CAPN3 systemically dosed CPN3KO mice show no detectable Calpain 3 protein expression in cardiac muscle.
Postive control (hCPN3) human quadriceps muscle lysate.

SUBSTITUTE SHEET (RULE 26)