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(54) **GENE THERAPY FOR COMBINED METHYLMALONIC ACIDEMIA/ACIDURIA AND HYPERHOMOCYSTEINEMIA/ HOMOCYSTINURIA, COBALAMIN C TYPE, AND DEFICIENCY OF MMACHC**

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(73) Assignee: **The United States of America, as represented by the Secretary, Department of Health and Human Servic**, Bethesda, MD (US)

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C12N 15/85	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 48/0066*** (2013.01); ***C07K 14/435*** (2013.01); ***A61P 43/00*** (2018.01); ***A61K 47/26*** (2013.01); ***C12N 15/67*** (2013.01); ***C12N 15/85*** (2013.01); *C12N 2750/14143* (2013.01)

(57) **ABSTRACT**

The present invention provides a synthetic MMACHC polynucleotide comprising a polynucleotide encoding MMACHC that is codon-optimized for expression in a human. Also provided is a polypeptide encoded by a synthetic MMACHC polynucleotide, an expression vector comprising a MMACHC gene sequence under the control of a chicken beta actin (CBA) promoter, and an expression vector comprising a synthetic MMACHC polynucleotide. Methods of treating cobalamin C deficiency and for detecting or tracking exogenous MMACHC are also provided.

Specification includes a Sequence Listing.

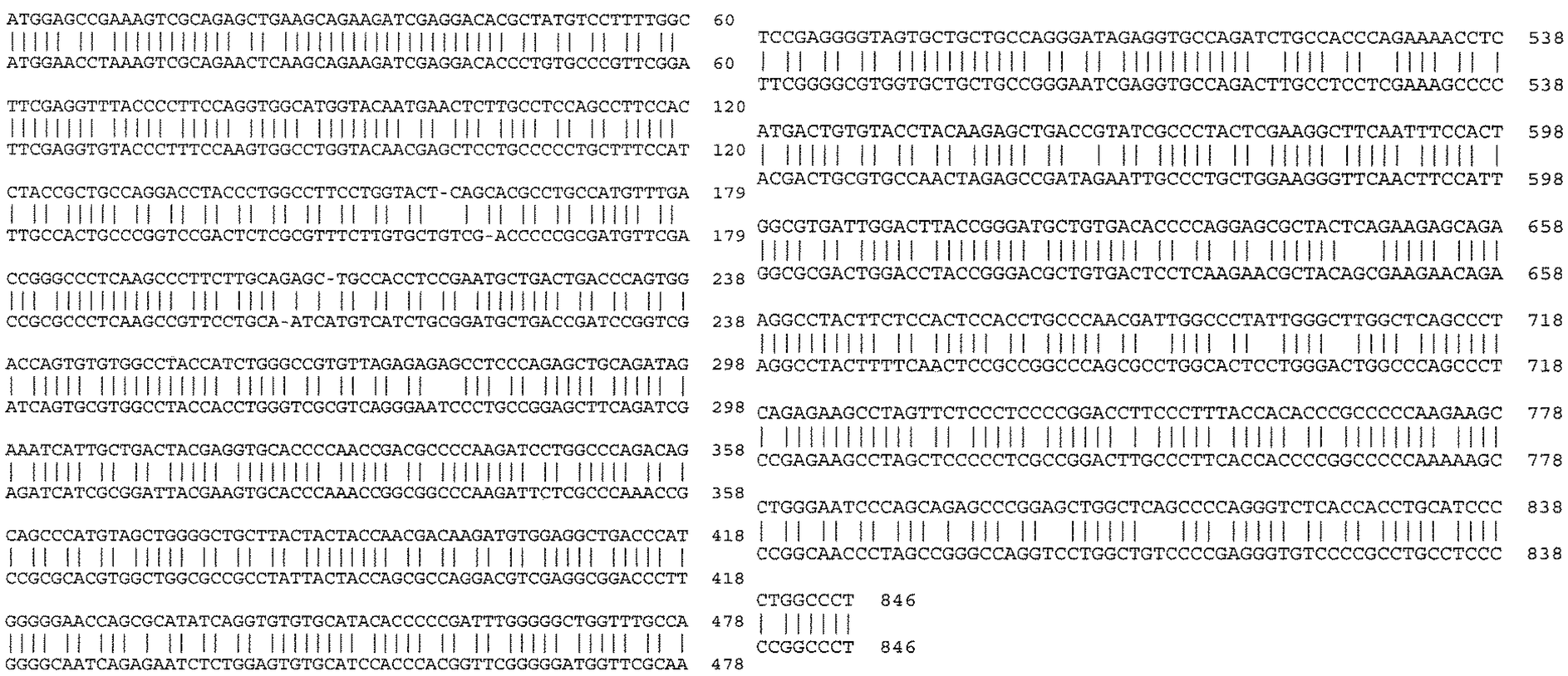


Figure 1A

```
ATGGAGCCGAAAGTCGCAGAGCTGAAGCAGAAGATCGAGGACACGCTATGTCCTTTTGGC  60
|||||  ||  ||||||||||||  ||  ||||||||||||||||||||||  ||  ||  ||  ||  ||
ATGGAACCTAAAGTCGCAGAACTCAAGCAGAAGATCGAGGACACCCTGTGCCCGTTCGGA  60

TTCGAGGTTTACCCCTTCCAGGTGGCATGGTACAATGAACTCTTGCCTCCAGCCTTCCAC  120
|||||||  |||||  |||||  |||||  ||||||||  ||  |||  ||||  ||  ||  |||||
TTCGAGGTGTACCCTTTCCAAGTGGCCTGGTACAACGAGCTCCTGCCCCCTGCTTTCCAT  120

CTACCGCTGCCAGGACCTACCCTGGCCTTCCTGGTACT-CAGCACGCCTGCCATGTTTGA  179
|  ||  |||||  ||  ||  ||  ||  ||  ||  ||  ||  ||    |  ||  ||  ||  |||||  ||
TTGCCACTGCCCGGTCCGACTCTCGCGTTTCTTGTGCTGTCG-ACCCCCGCGATGTTCGA  179

CCGGGCCCTCAAGCCCTTCTTGCAGAGC-TGCCACCTCCGAATGCTGACTGACCCAGTGG  238
|||  ||||||||||||  |||  ||||  |  |  ||  ||  ||  ||  ||||||||  ||  ||  ||  |
CCGCGCCCTCAAGCCGTTCCTGCA-ATCATGTCATCTGCGGATGCTGACCGATCCGGTCG  238

ACCAGTGTGTGGCCTACCATCTGGGCCGTGTTAGAGAGAGCCTCCCAGAGCTGCAGATAG  298
|  |||||  ||||||||||||  |||||  ||  ||  ||  ||    |||  ||  |||||  |||||  |
ATCAGTGCGTGGCCTACCACCTGGGTCGCGTCAGGGAATCCCTGCCGGAGCTTCAGATCG  298

AAATCATTGCTGACTACGAGGTGCACCCCAACCGACGCCCCAAGATCCTGGCCCAGACAG  358
|  |||||  ||  ||  |||||  ||||||||  |||||  ||  ||||||||  ||  |||||  ||  |
AGATCATCGCGGATTACGAAGTGCACCCAAACCGGCGGCCCAAGATTCTCGCCCAAACCG  358

CAGCCCATGTAGCTGGGGCTGCTTACTACTACCAACGACAAGATGTGGAGGCTGACCCAT  418
|  ||  ||  ||  |||||  ||  ||  ||  ||||||||  ||  ||  ||  ||  |||||  |||||  |
CCGCGCACGTGGCTGGCGCCGCCTATTACTACCAGCGCCAGGACGTCGAGGCGGACCCTT  418

GGGGGAACCAGCGCATATCAGGTGTGTGCATACACCCCCGATTTGGGGGCTGGTTTGCCA  478
||||  ||  |||  |  ||  ||  ||  ||||||||  |||||  ||  ||  |||||  |||||  ||  |
GGGGCAATCAGAGAATCTCTGGAGTGTGCATCCACCCACGGTTCGGGGGATGGTTCGCAA  478
```

Figure 1B

```
TCCGAGGGGTAGTGCTGCTGCCAGGGATAGAGGTGCCAGATCTGCCACCCAGAAAACCTC  538
| || || || |||||||||||| || || |||||||||||  |||| ||  |||| || |
TTCGGGGCGTGGTGCTGCTGCCGGGAATCGAGGTGCCAGACTTGCCTCCTCGAAAGCCCC  538

ATGACTGTGTACCTACAAGAGCTGACCGTATCGCCCTACTCGAAGGCTTCAATTTCCACT  598
| ||||| || || || ||||| ||  | || ||||| || ||||| ||||| ||||| |
ACGACTGCGTGCCAACTAGAGCCGATAGAATTGCCCTGCTGGAAGGGTTCAACTTCCATT  598

GGCGTGATTGGACTTACCGGGATGCTGTGACACCCCAGGAGCGCTACTCAGAAGAGCAGA  658
||||  ||  |||||  |||||||| |||||||| || || || ||||||   |||||  ||||
GGCGCGACTGGACCTACCGGGACGCTGTGACTCCTCAAGAACGCTACAGCGAAGAACAGA  658

AGGCCTACTTCTCCACTCCACCTGCCCAACGATTGGCCCTATTGGGCTTGGCTCAGCCCT  718
||||||||||  || |||||  || |||||  ||  ||||  ||  ||||  ||||  |||||||
AGGCCTACTTTTCAACTCCGCCGGCCCAGCGCCTGGCACTCCTGGGACTGGCCCAGCCCT  718

CAGAGAAGCCTAGTTCTCCCTCCCCGGACCTTCCCTTTACCACACCCGCCCCCAAGAAGC  778
| ||||||||||||  || |||||  ||||||  | |||||  |||||  || |||||||| ||||
CCGAGAAGCCTAGCTCCCCCTCGCCGGACTTGCCCTTCACCACCCCGGCCCCCAAAAAGC  778

CTGGGAATCCCAGCAGAGCCCGGAGCTGGCTCAGCCCCAGGGTCTCACCACCTGCATCCC  838
| ||  ||  ||  |||  |  |||  ||  ||||||   |||  |||||  ||  ||  |||||  ||||
CCGGCAACCCTAGCCGGGCCAGGTCCTGGCTGTCCCCGAGGGTGTCCCCGCCTGCCTCCC  838

CTGGCCCT  846
| ||||||
CCGGCCCT  846
```

Figure 2

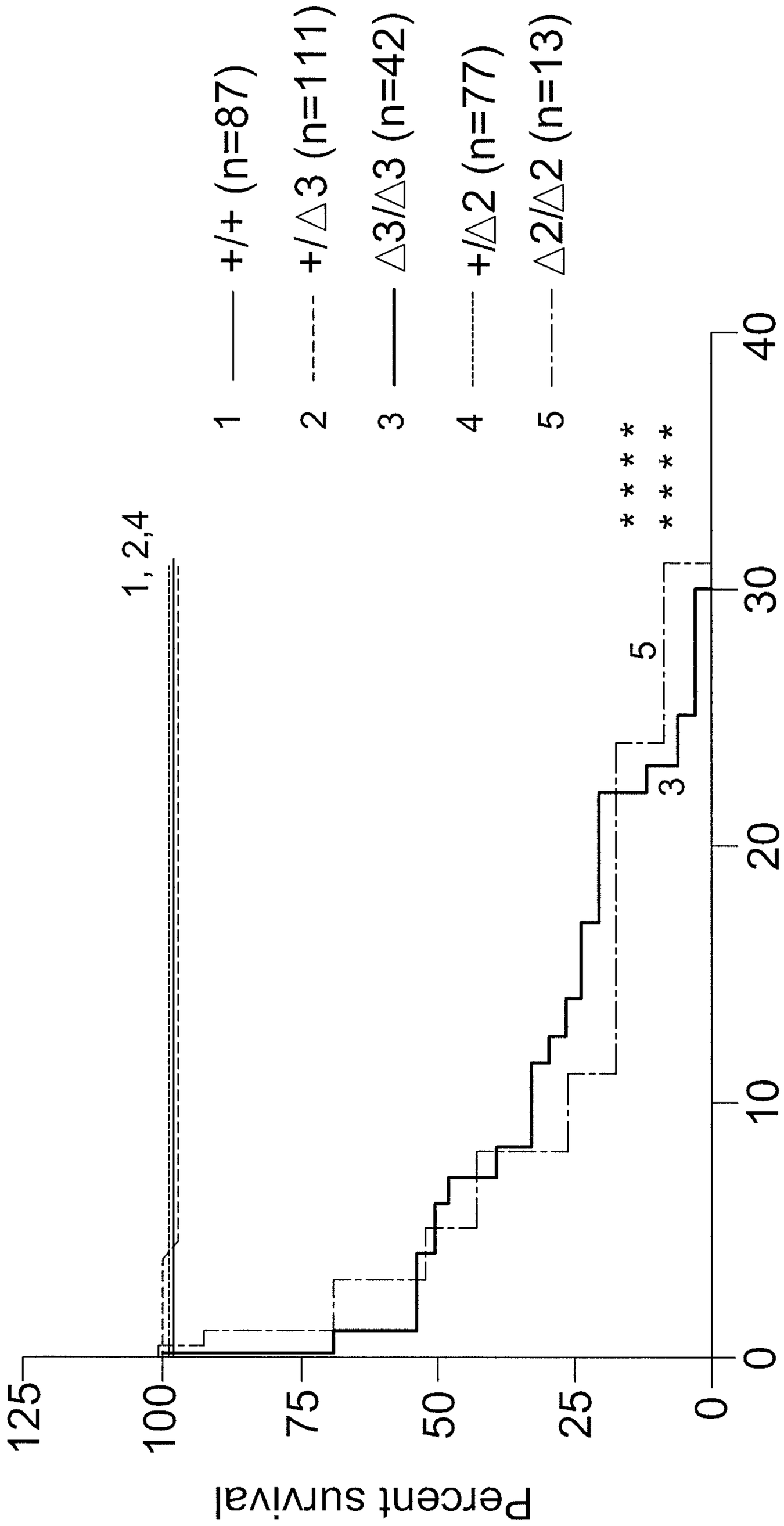

+/+ (n=87)
+/△3 (n=111)
△3/△3 (n=42)
+/△2 (n=77)
△2/△2 (n=13)
1
2
3
4
5
1, 2,4
40
* * * *
* * * *
5
3
30
20
10
0
Age (days)
125
100
75
50
25
0
Percent survival

Figure 3

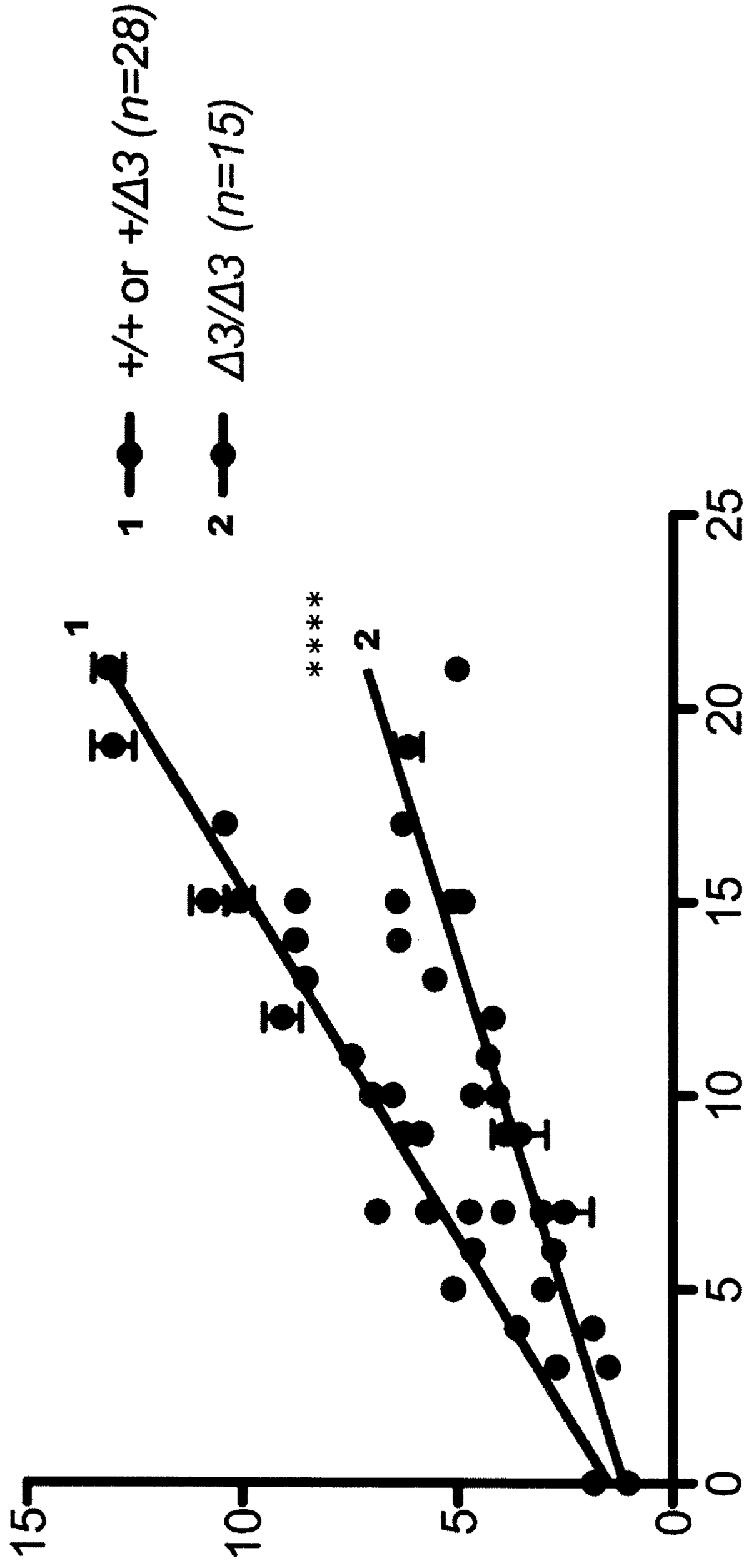


Figure 4A

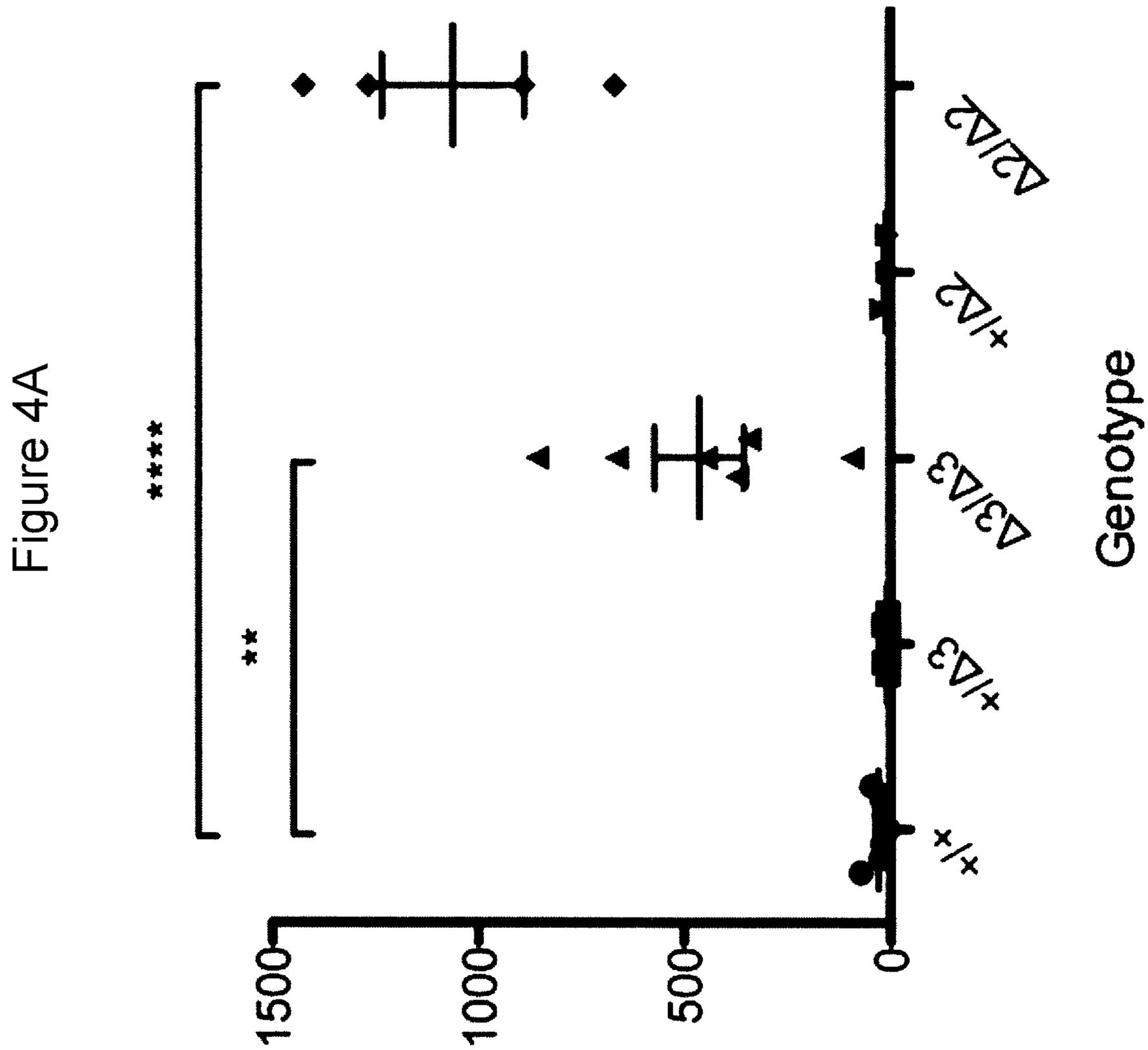
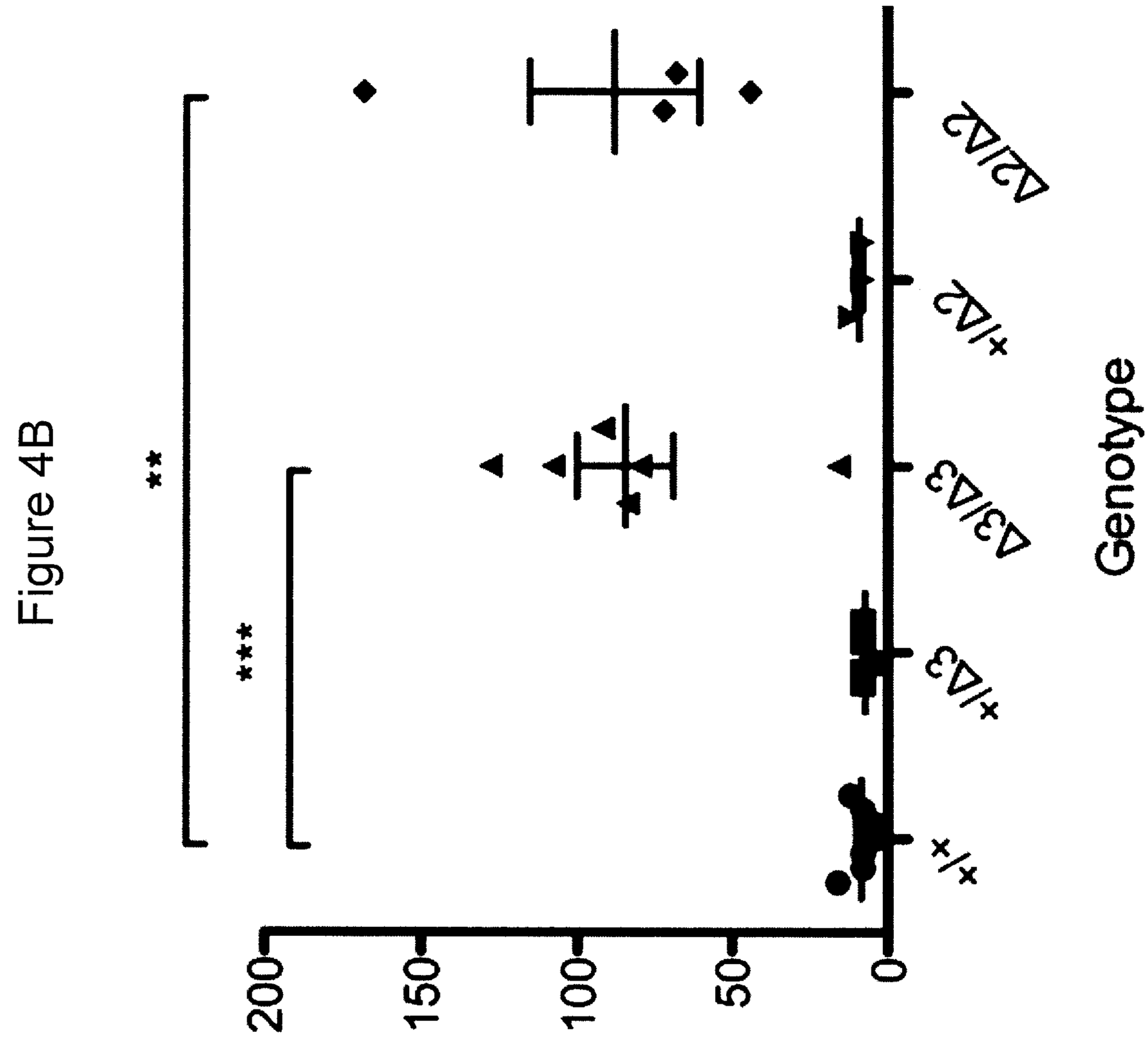

****
**
Methylmalonic acid (uM)
150
100
50
x/x
Δ3/x
Δ3/Δ3
Δ2/x
Δ2/Δ2
Genotype

Figure 4B


**
***
Homocysteine (uM)
200
150
100
50
0
x/x
ε3/x
ε3/ε3
ε2/x
ε2/ε2
Genotype

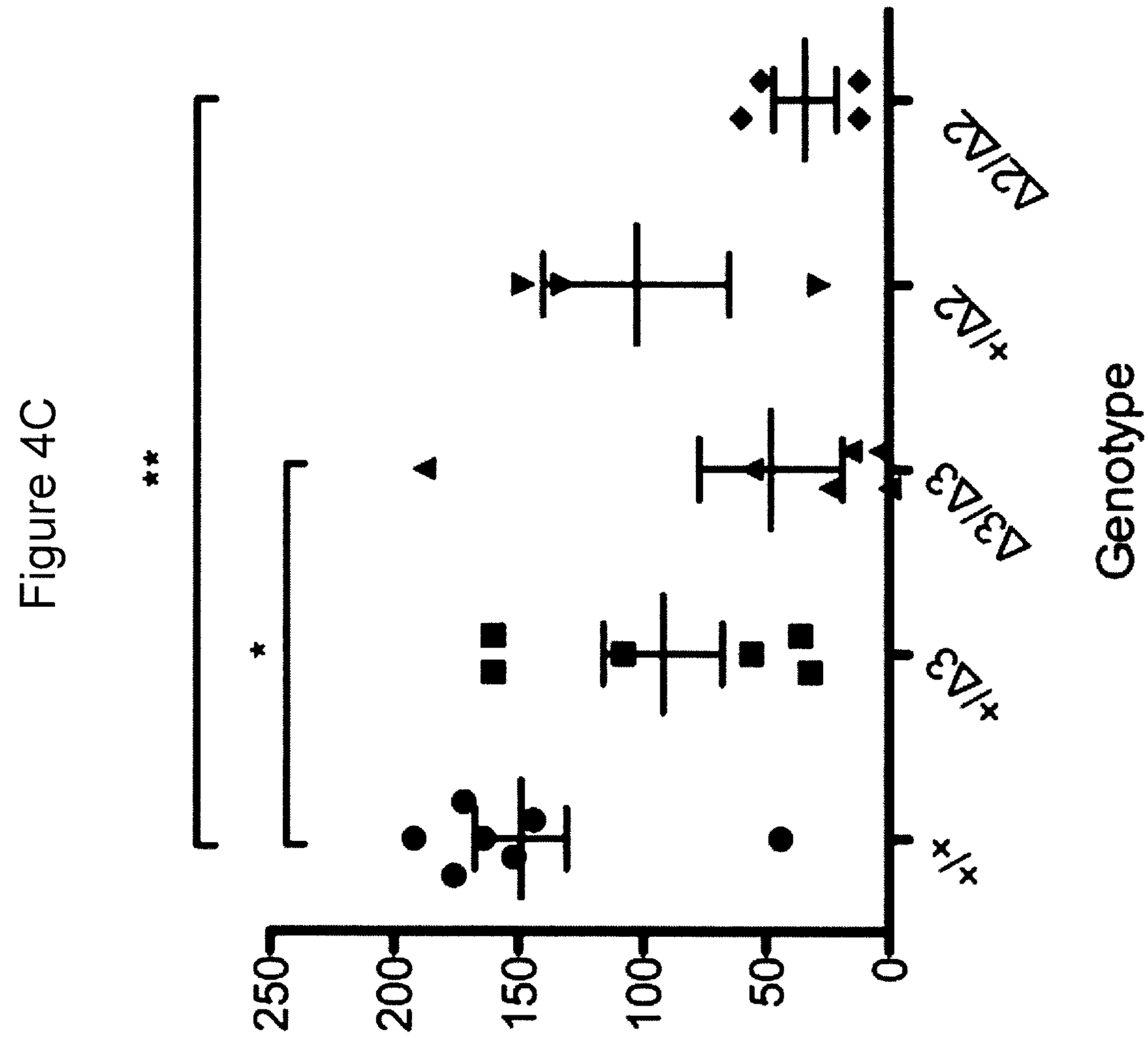


Figure 4C

Figure 5

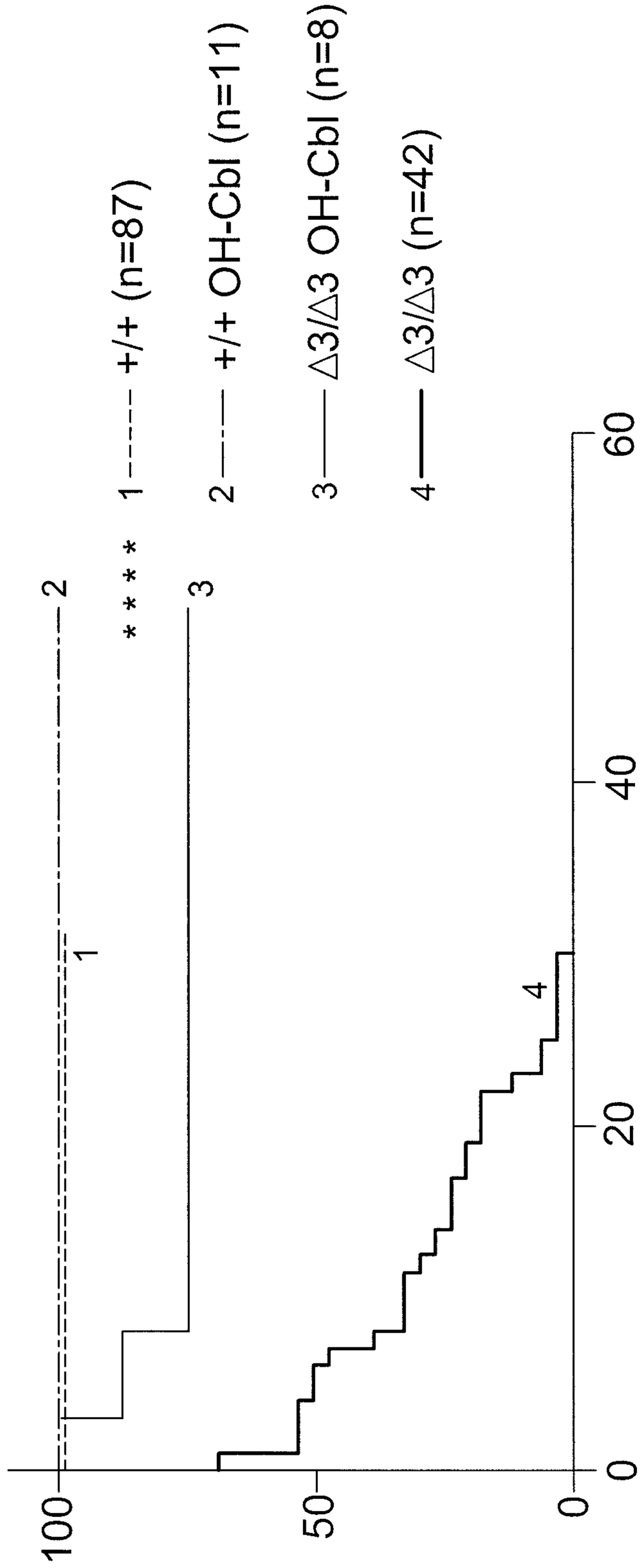

1 +/+ (n=87)
2 +/+ OH-Cbl (n=11)
3 Δ3/Δ3 OH-Cbl (n=8)
4 Δ3/Δ3 (n=42)
Percent survival
100
50
0
0
20
40
60
Days

Figure 6

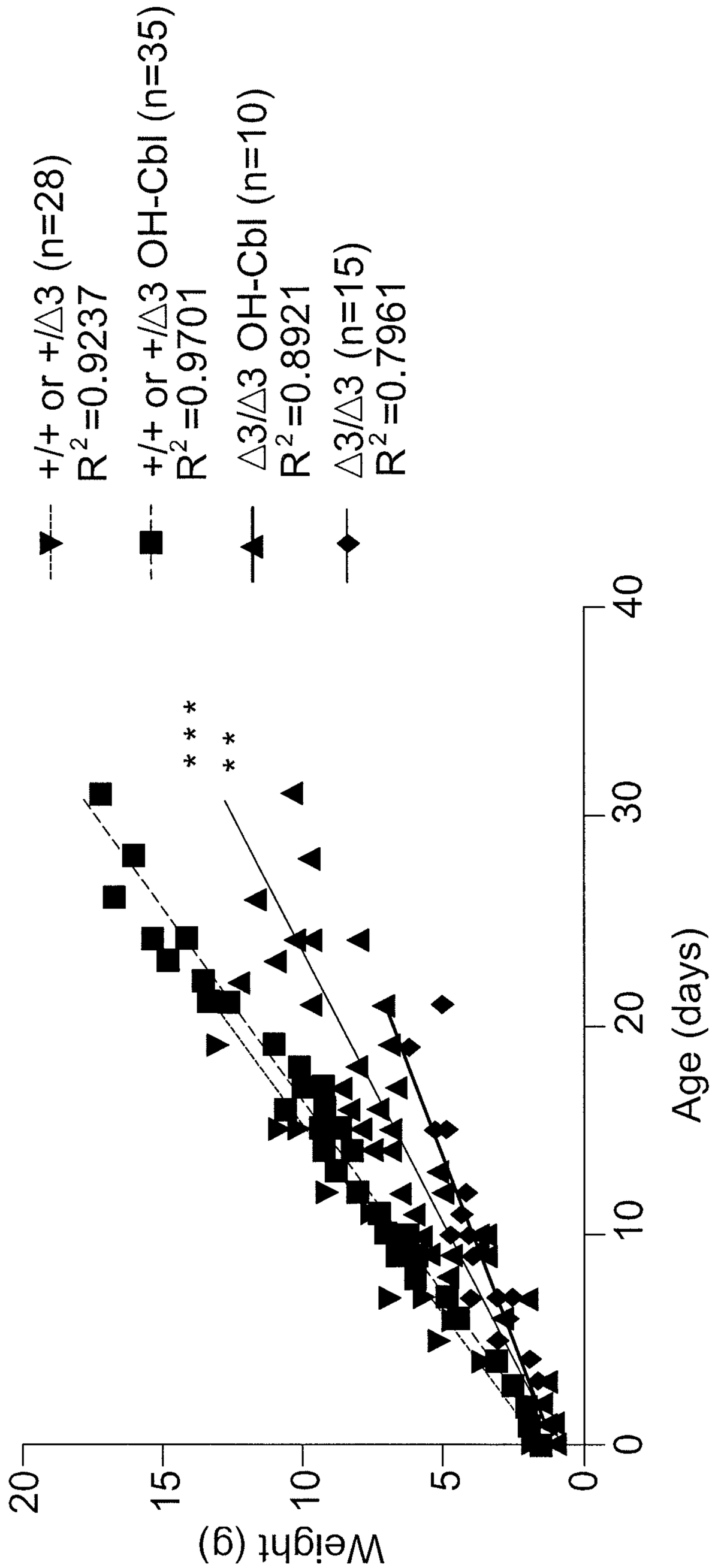

+/+ or +/Δ3 (n=28)
R²=0.9237
+/+ or +/Δ3 OH-Cbl (n=35)
R²=0.9701
Δ3/Δ3 OH-Cbl (n=10)
R²=0.8921
Δ3/Δ3 (n=15)
R²=0.7961
* * * * * *
Weight (g)
0
5
10
15
20
Age (days)
0
10
20
30
40

Figure 7A

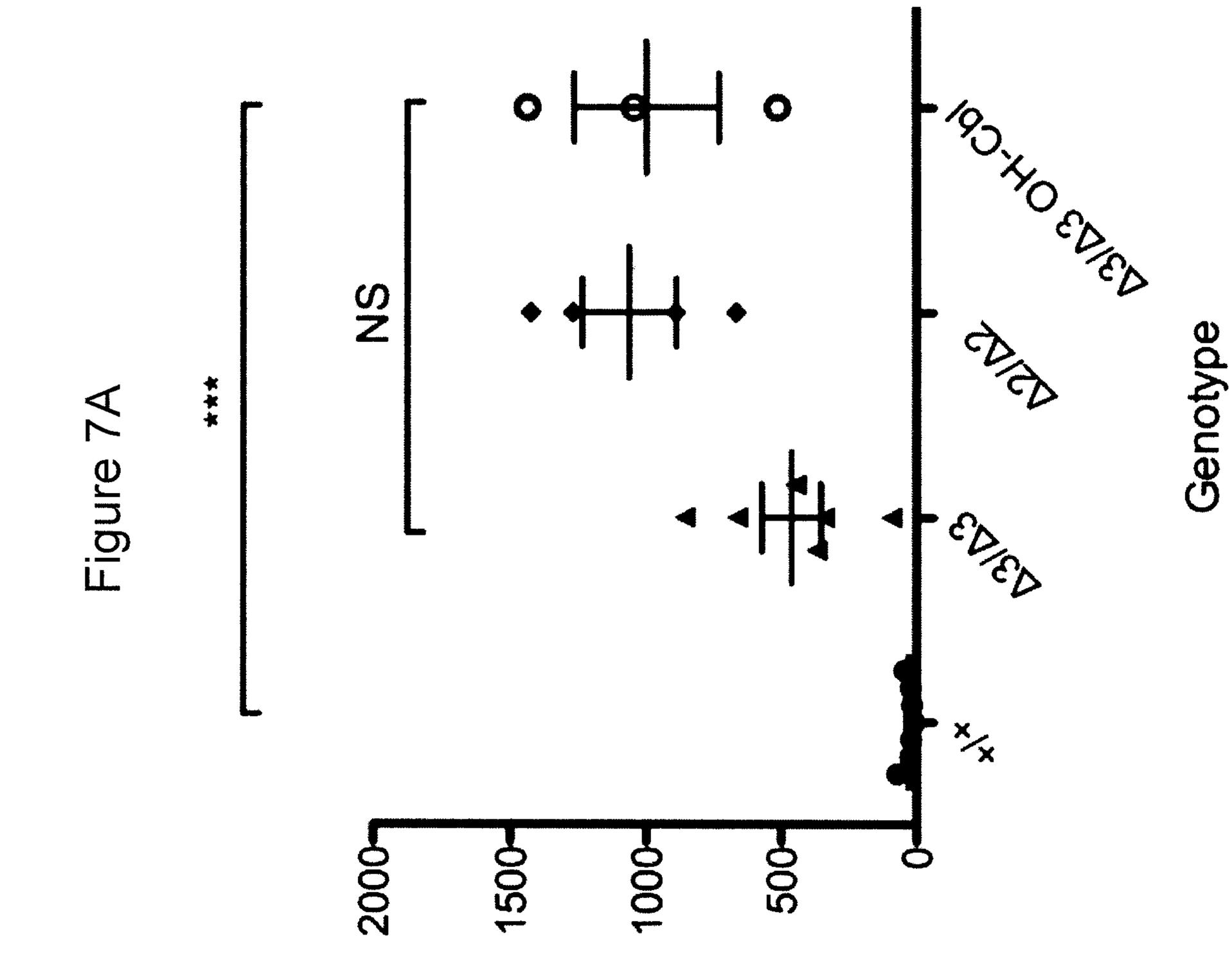

***
NS
Methylmalonic acid (uM)
0
5
10
15
20
+/+
Δ3/Δ3
Δ2/Δ2
Δ3/Δ3 OH-Cbl
Genotype

Figure 7B

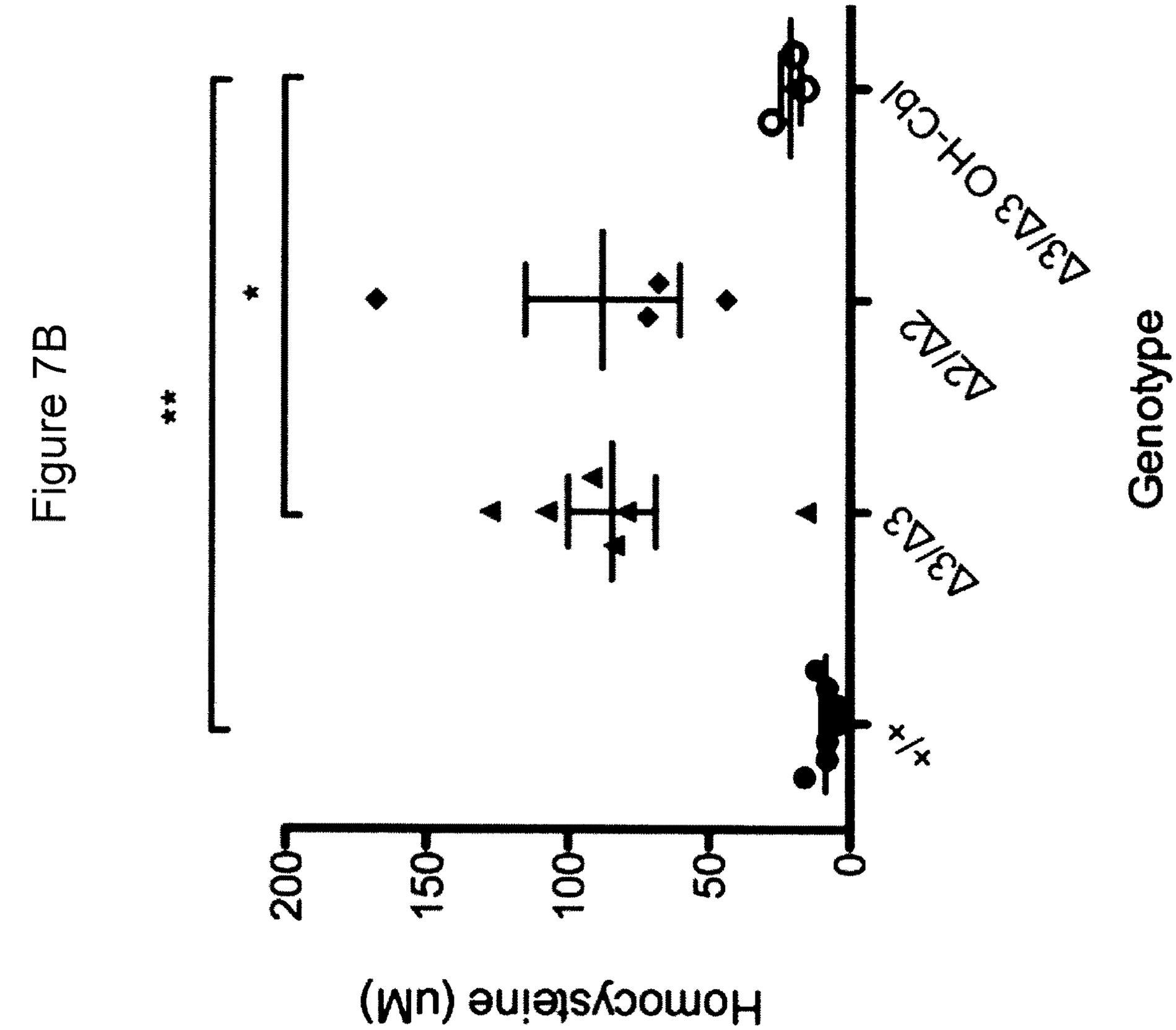

Homocysteine (uM)
200
150
100
50
0
+/+
Δ3/Δ3
Δ2/Δ2
Δ3/Δ3 OH-Cbl
Genotype
**
*

Figure 8

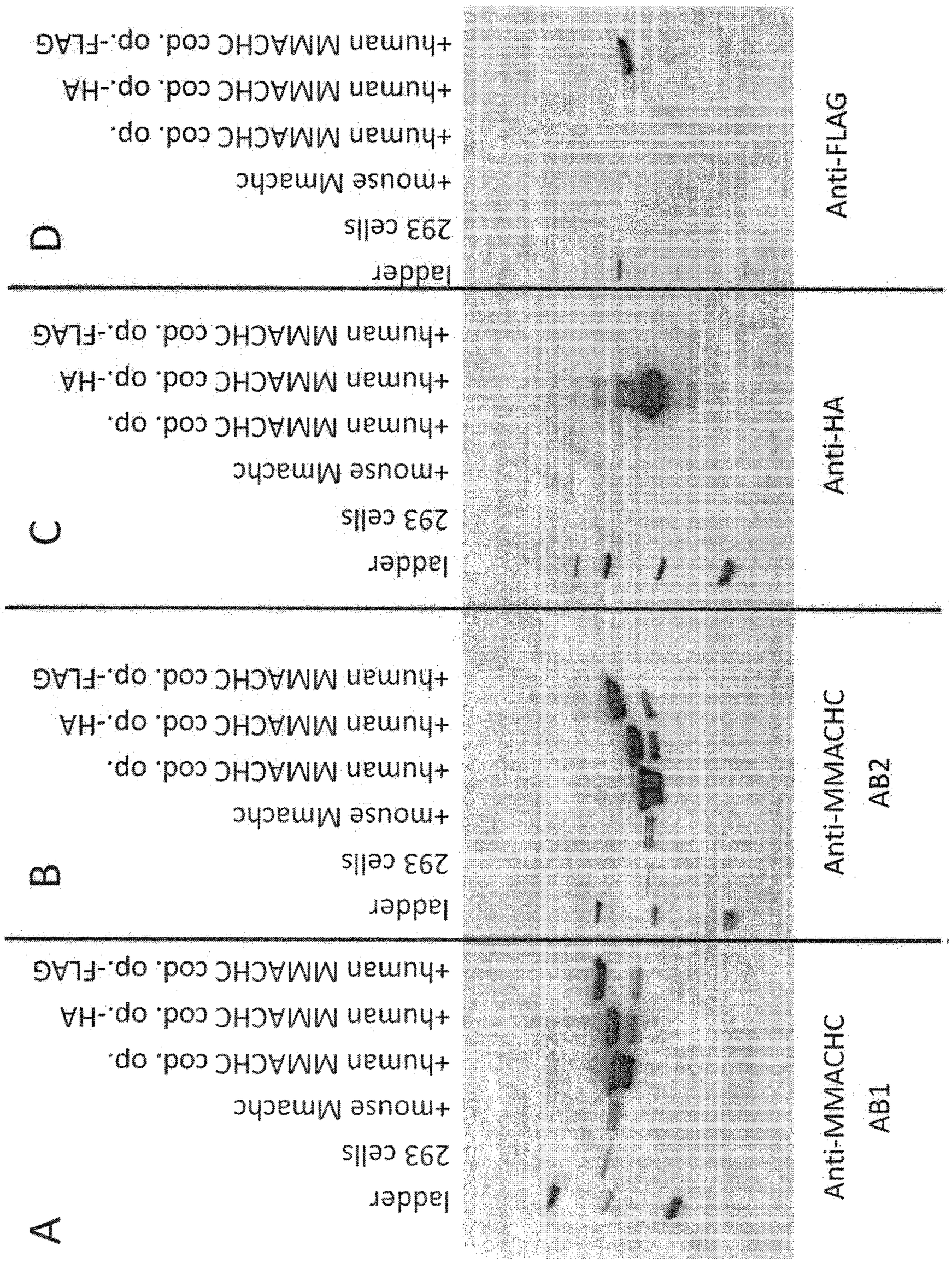

A
ladder
293 cells
+mouse Mmachc
+human MMACHC cod. op.
+human MMACHC cod. op.-HA
+human MMACHC cod. op.-FLAG
Anti-MMACHC
AB1
B
ladder
293 cells
+mouse Mmachc
+human MMACHC cod. op.
+human MMACHC cod. op.-HA
+human MMACHC cod. op.-FLAG
Anti-MMACHC
AB2
C
ladder
293 cells
+mouse Mmachc
+human MMACHC cod. op.
+human MMACHC cod. op.-HA
+human MMACHC cod. op.-FLAG
Anti-HA
D
ladder
293 cells
+mouse Mmachc
+human MMACHC cod. op.
+human MMACHC cod. op.-HA
+human MMACHC cod. op.-FLAG
Anti-FLAG

Figure 9A

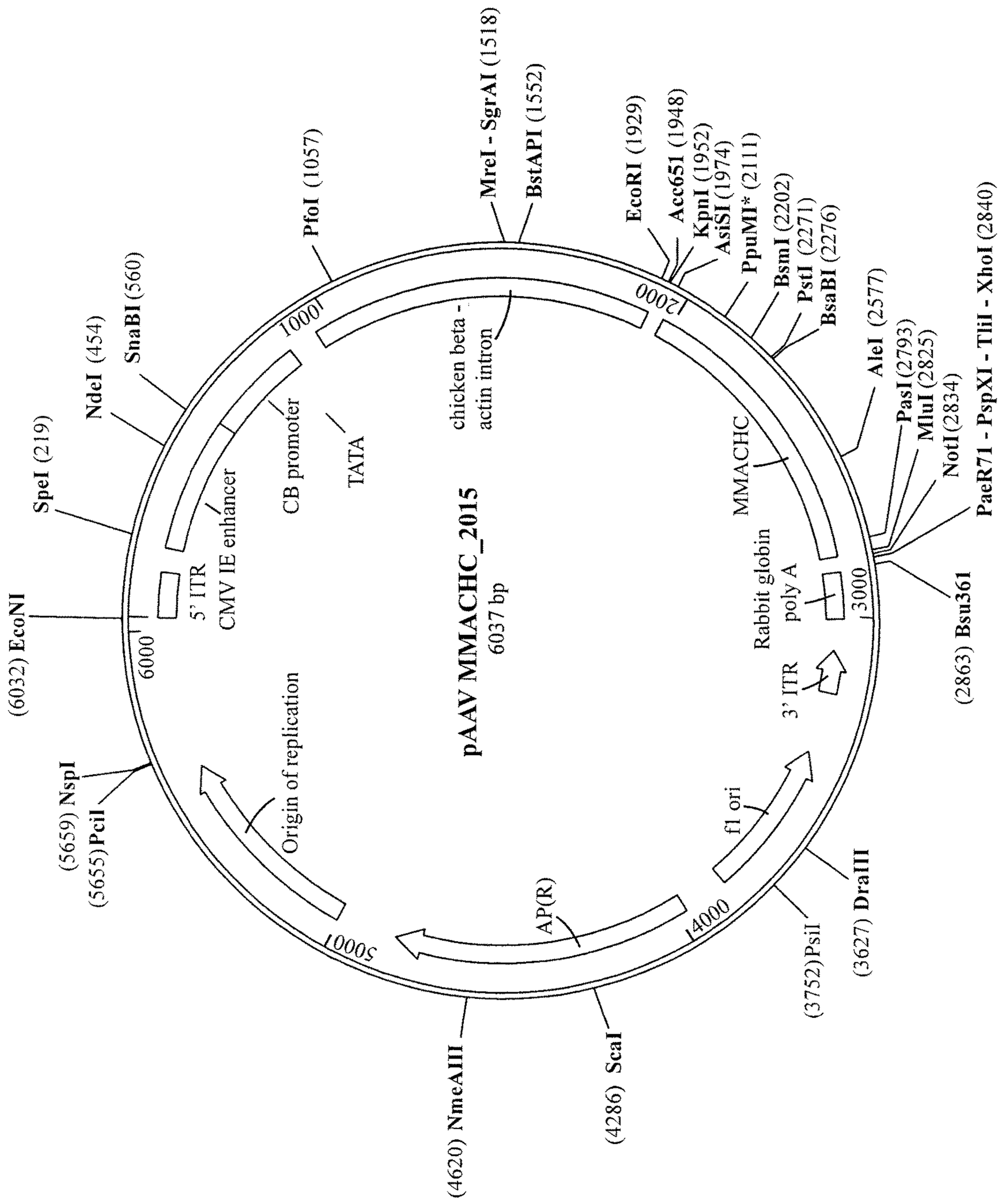

pAAV MMACHC_2015
6037 bp
1000
2000
3000
4000
5000
6000
5' ITR
CMV IE enhancer
CB promoter
TATA
chicken beta - actin intron
MMACHC
Rabbit globin poly A
3' ITR
AP(R)
f1 ori
Origin of replication
(6032) EcoNI
SpeI (219)
NdeI (454)
SnaBI (560)
PfoI (1057)
MreI - SgrAI (1518)
BstAPI (1552)
EcoRI (1929)
Acc65I (1948)
KpnI (1952)
AsiSI (1974)
PpuMI* (2111)
BsmI (2202)
PstI (2271)
BsaBI (2276)
AleI (2577)
PasI (2793)
MluI (2825)
NotI (2834)
PaeR71 - PspXI - TliI - XhoI (2840)
(2863) Bsu361
(3627) DraIII
(3752) PsiI
(4286) ScaI
(4620) NmeAIII
(5655) PciI
(5659) NspI

Figure 9B

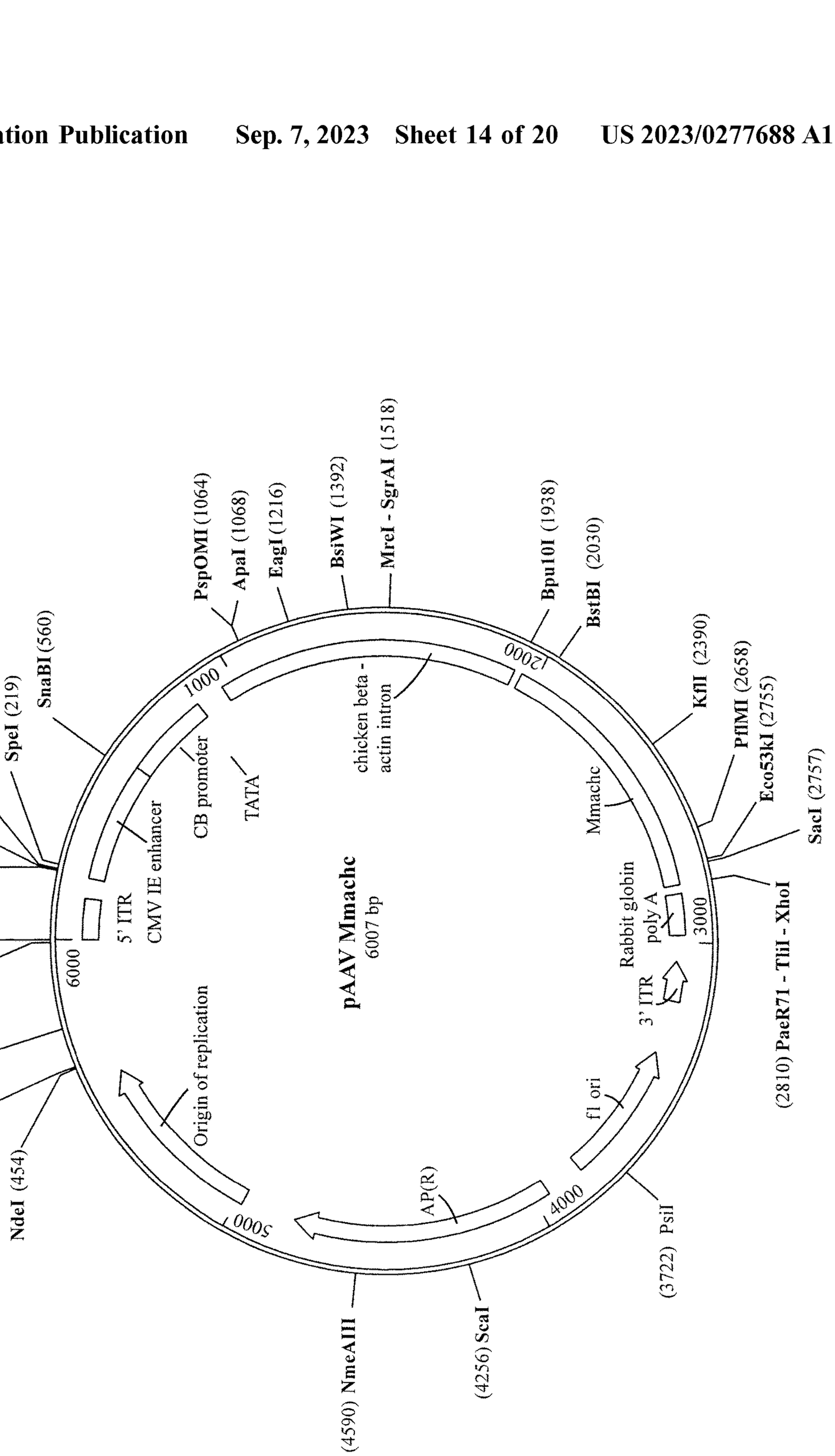

pAAV Mmachc
6007 bp
5' ITR
CMV IE enhancer
CB promoter
TATA
chicken beta - actin intron
Mmachc
Rabbit globin poly A
3' ITR
f1 ori
AP(R)
Origin of replication
1000
2000
3000
4000
5000
6000
EcoNI (6002)
SalI (202)
AccI (203)
HincII (204)
SpeI (219)
SnaBI (560)
PspOMI (
ApaI (10
EagI (1
BsiWI (1392)
MreI - SgrAI (1518)
Bpu10I (1
BstBI (2030)
KfiI (2390)
PflMI (2658)
Eco53kI (2755)
SacI (2757)
(2810) PaeR71 - TliI - XhoI
(3722) PsiI
(4256) ScaI
(4590) NmeAIII
NdeI (454)
NdeI (454)
NdeI (454)
NdeI (454)

Figure 9C

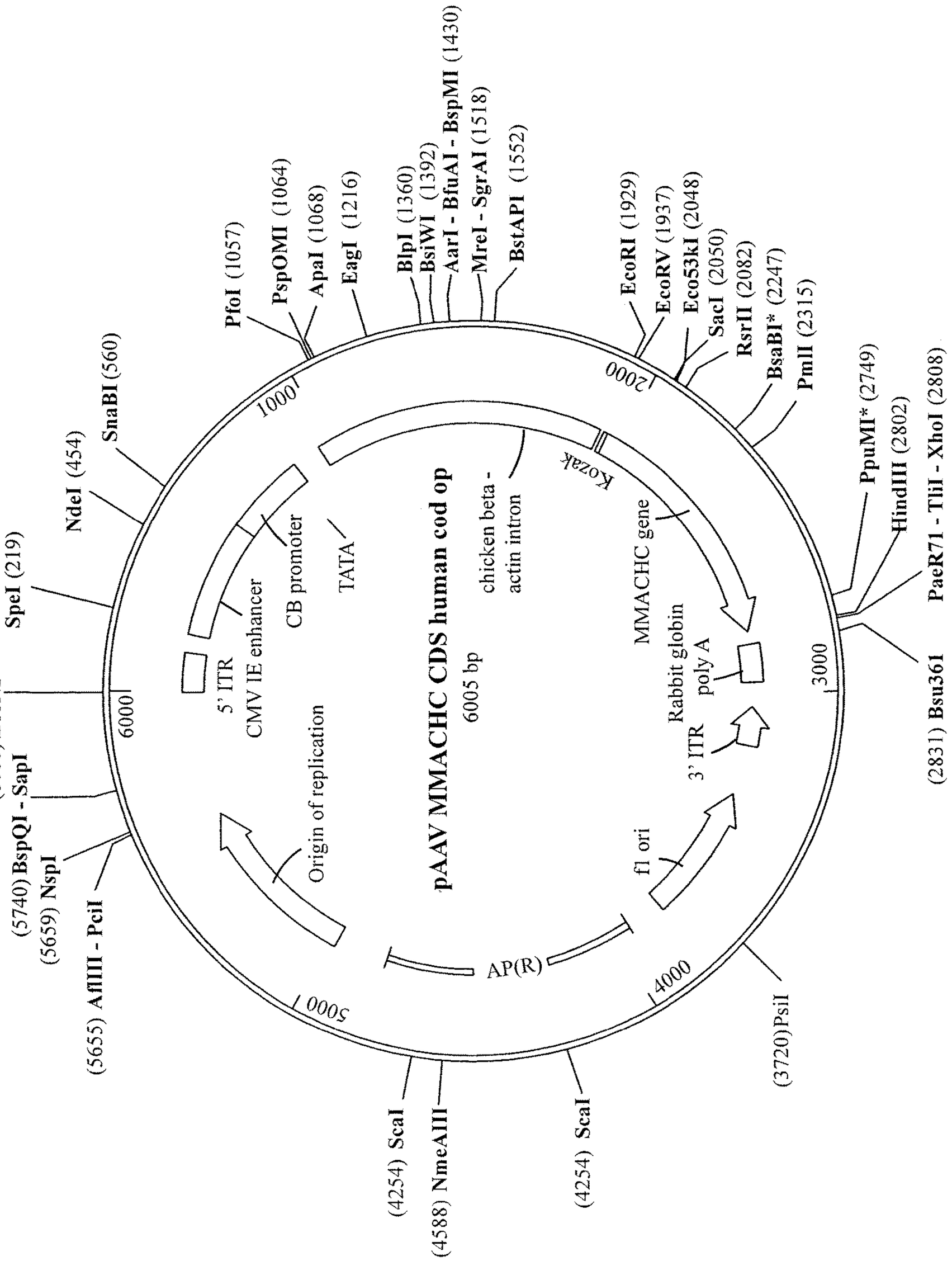

pAAV MMACHC CDS human cod op
6005 bp
5' ITR
CMV IE enhancer
CB promoter
TATA
chicken beta - actin intron
Kozak
MMACHC gene
Rabbit globin poly A
3' ITR
f1 ori
Origin of replication
AP(R)
1000
2000
3000
4000
5000
6000
(6000) EcoNI
SpeI (219)
NdeI (454)
SnaBI (560)
(5740) BspQI - SapI
(5659) NspI
(5655) AflIII - PciI
(4254) ScaI
(4588) NmeAIII
(4254) ScaI
(3720)PsiI
PfoI (1057)
PspOMI (1064)
ApaI (1068)
EagI (1216)
BlpI (1360)
BsiWI (1392)
AarI - BfuAI - BspMI (1430)
MreI - SgrAI (1518)
BstAPI (1552)
EcoRI (1929)
EcoRV (1937)
Eco53kI (2048)
SacI (2050)
RsrII (2082)
BsaBI* (2
PmlI (231
PpuMI* (2749)
HindIII (2802)
(2831) Bsu361
PaeR71 - TliI - XhoI (2808)

Figure 9D

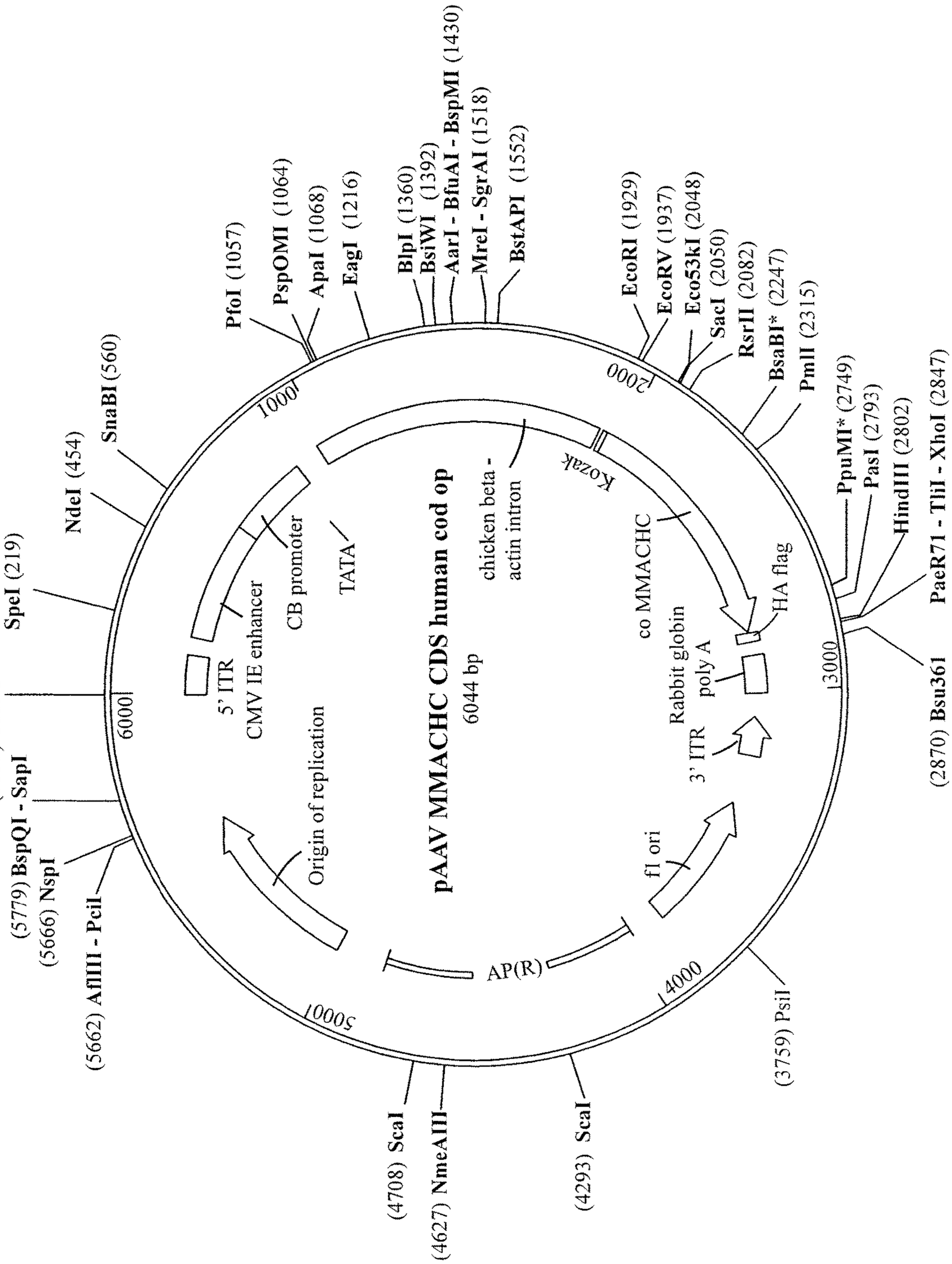

pAAV MMACHC CDS human cod op
6044 bp
5' ITR
CMV IE enhancer
CB promoter
TATA
chicken beta - actin intron
Kozak
co MMACHC
HA flag
Rabbit globin poly A
3' ITR
Origin of replication
f1 ori
AP(R)
1000
2000
3000
4000
5000
6000
(6039) EcoNI
BspQI - SapI
(5779) BspQI - SapI
(5666) NspI
(5662) AflIII - PciI
SpeI (219)
NdeI (454)
SnaBI (560)
PfoI (1057)
PspOMI (1064)
ApaI (1068)
EagI (1216)
BlpI (1360)
BsiWI (1392)
AarI - BfuAI - BspMI (1430)
MreI - SgrAI (1518)
BstAPI (1552)
EcoRI (1929)
EcoRV (1937)
Eco53kI (2048)
SacI (2050)
RsrII (2082)
BsaBI* (2247)
PmlI (2315)
PpuMI* (2749)
PasI (2793)
HindIII (2802)
(2870) Bsu361
PaeR71 - TliI - XhoI (2847)
(3759) PsiI
(4293) ScaI
(4627) NmeAIII
(4708) ScaI

Figure 9E

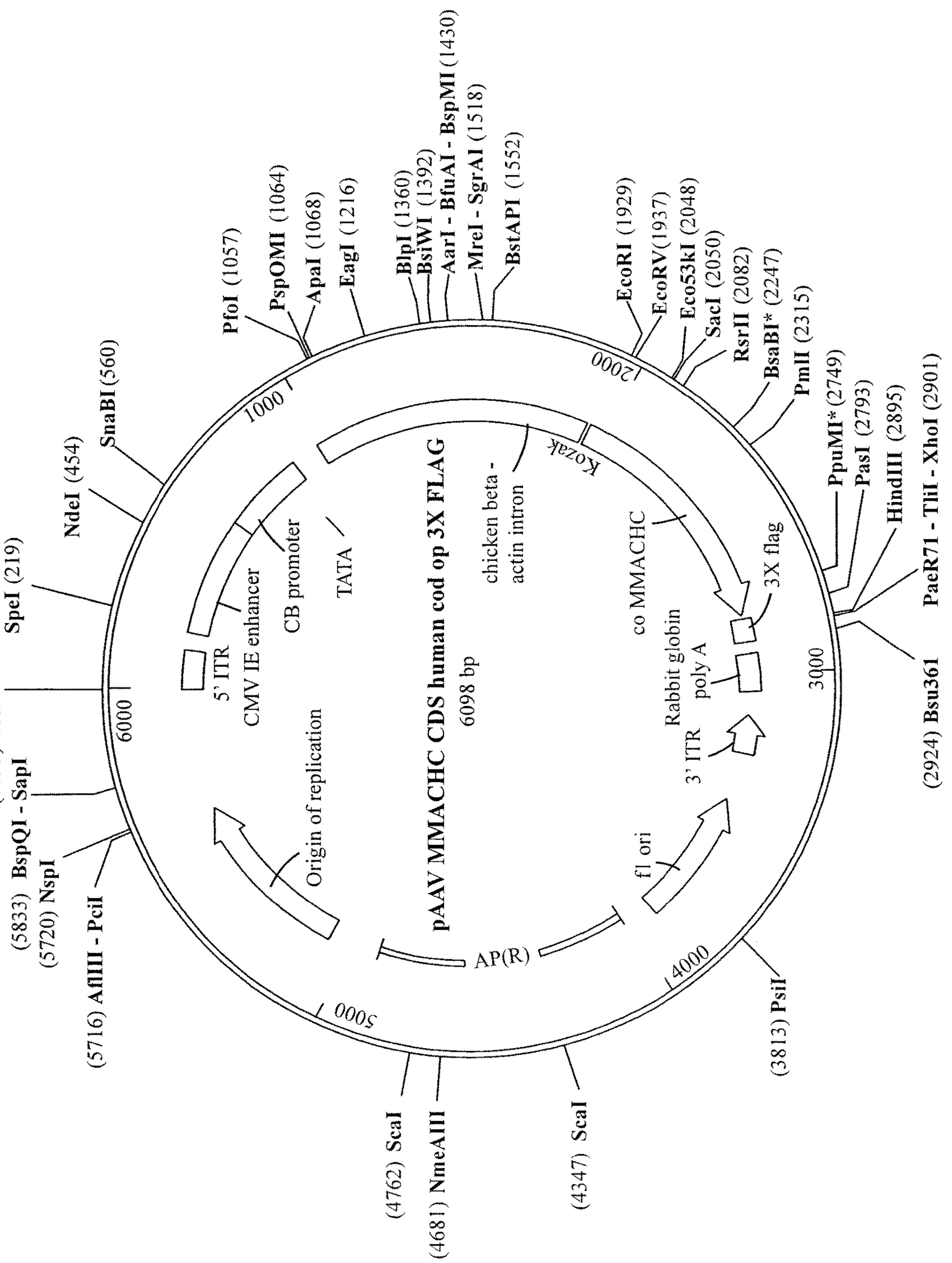

pAAV MMACHC CDS human cod op 3X FLAG
6098 bp
1000
2000
3000
4000
5000
6000
5' ITR
CMV IE enhancer
CB promoter
TATA
chicken beta - actin intron
Kozak
co MMACHC
3X flag
Rabbit globin poly A
3' ITR
f1 ori
Origin of replication
AP(R)
SpeI (219)
NdeI (454)
SnaBI (560)
PfoI (1057)
PspOMI (1064)
ApaI (1068)
EagI (1216)
BlpI (1360)
BsiWI (1392)
AarI - BfuAI - BspMI (1430)
MreI - SgrAI (1518)
BstAPI (1552)
EcoRI (1929)
EcoRV(1937)
Eco53kI (2048)
SacI (2050)
RsrII (2082)
BsaBI* (2247)
PmlI (2315)
PpuMI* (2749)
PasI (2793)
HindIII (2895)
PaeR71 - TliI - XhoI (2901)
(2924) Bsu361
(3813) PsiI
(4347) ScaI
(4681) NmeAIII
(4762) ScaI
(5716) AflIII - PciI
(5720) NspI
(5833) BspQI - SapI
(6093) EcoNI

Figure 10

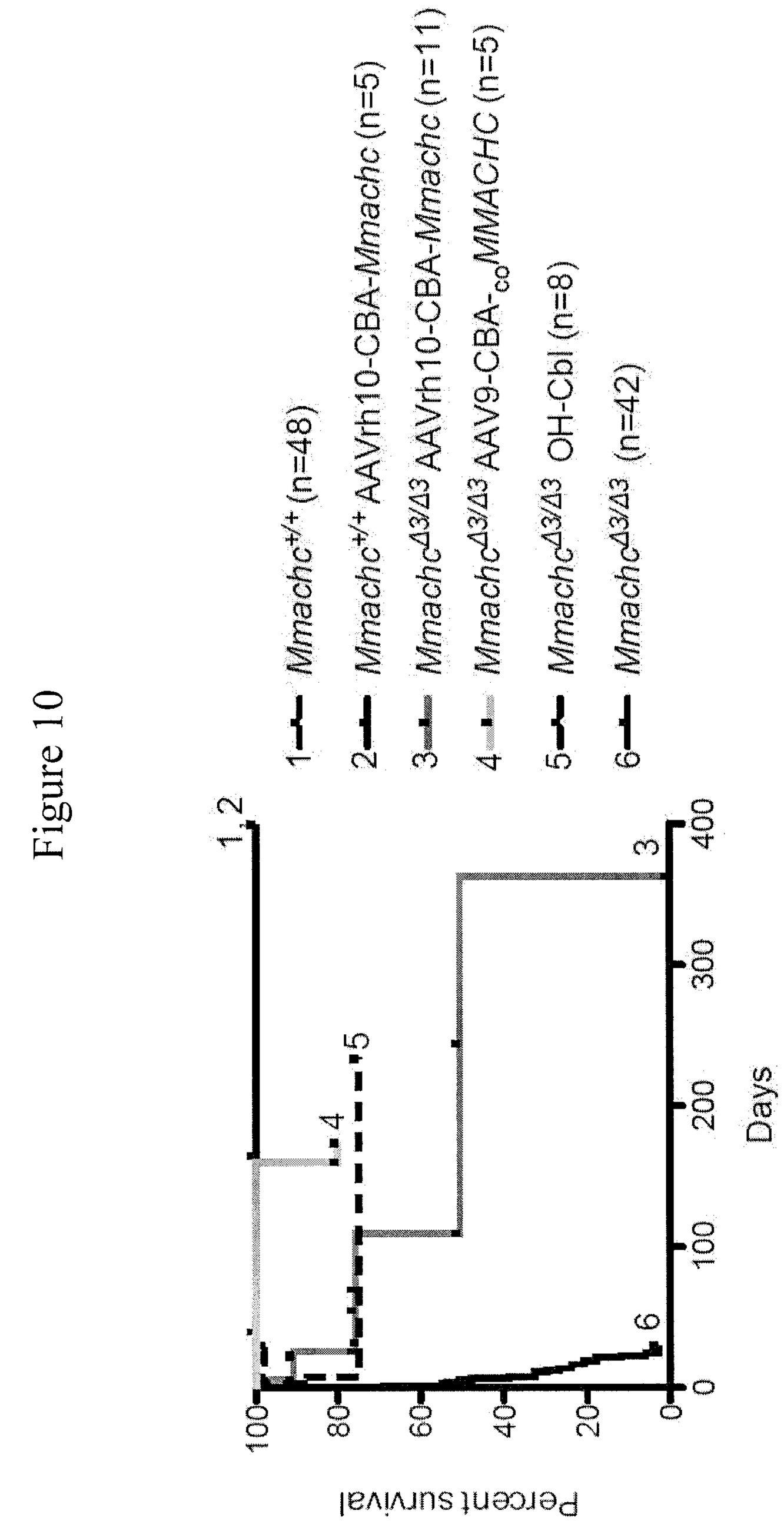

1 Mmachc+/+ (n=48)
2 Mmachc+/+ AAVrh10-CBA-Mmachc (n=5)
3 Mmachc Δ3/Δ3 AAVrh10-CBA-Mmachc (n=11)
4 Mmachc Δ3/Δ3 AAV9-CBA-co MMACHC (n=5)
5 Mmachc Δ3/Δ3 OH-Cbl (n=8)
6 Mmachc Δ3/Δ3 (n=42)
Percent survival
Days
100
80
60
40
20
0
0
100
200
300
400
1, 2
3
4
5
6

Figure 11

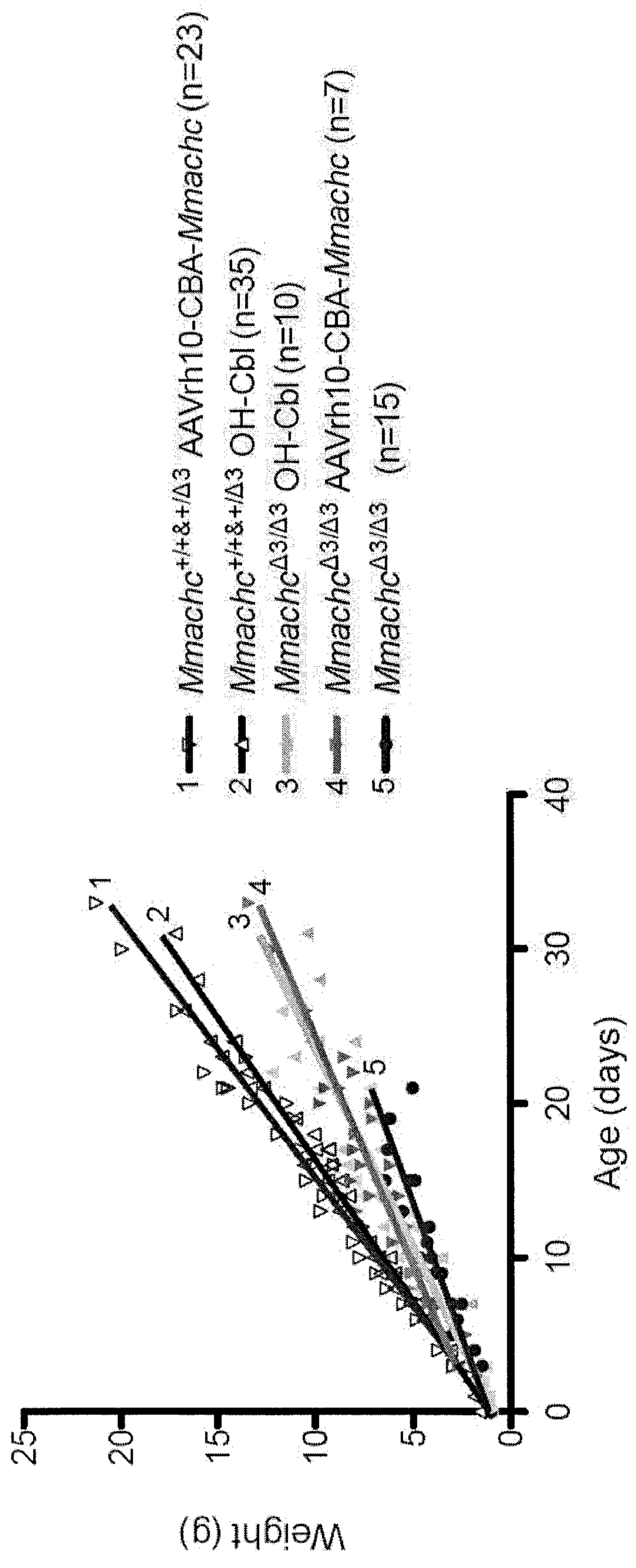
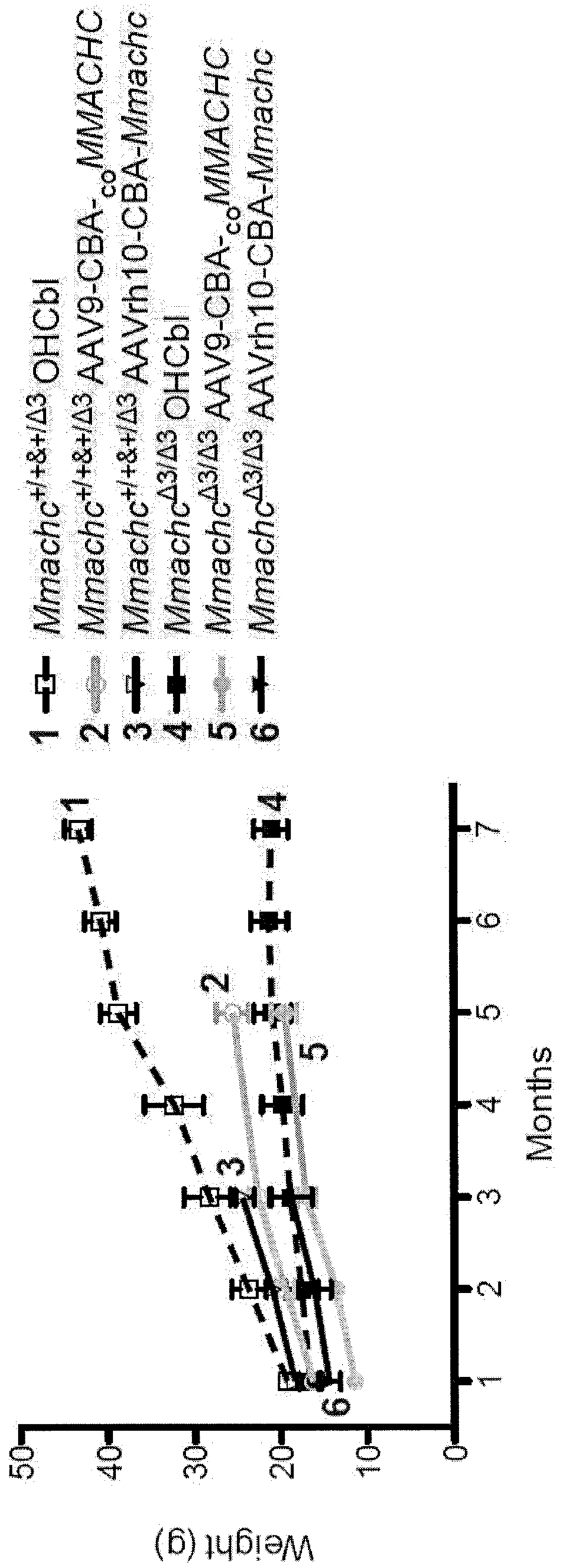

1 Mmachc+/+&+/Δ3 AAVrh10-CBA-Mmachc (n=23)
2 Mmachc+/+&+/Δ3 OH-Cbl (n=35)
3 MmachcΔ3/Δ3 OH-Cbl (n=10)
4 MmachcΔ3/Δ3 AAVrh10-CBA-Mmachc (n=7)
5 MmachcΔ3/Δ3 (n=15)
Weight (g)
25
20
15
10
5
0
Age (days)
0
10
20
30
40

Figure 12


1 Mmachc+/+&+/Δ3 OHCbl
2 Mmachc+/+&+/Δ3 AAV9-CBA-coMMACHC
3 Mmachc+/+&+/Δ3 AAVrh10-CBA-Mmachc
4 MmachcΔ3/Δ3 OHCbl
5 MmachcΔ3/Δ3 AAV9-CBA-coMMACHC
6 MmachcΔ3/Δ3 AAVrh10-CBA-Mmachc
Weight (g)
Months
0
10
20
30
40
50
1
2
3
4
5
6
7

GENE THERAPY FOR COMBINED METHYLMALONIC ACIDEMIA/ACIDURIA AND HYPERHOMOCYSTEINEMIA/HOMOCYSTINURIA, COBALAMIN C TYPE, AND DEFICIENCY OF MMACHC

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application is a divisional application of U.S. patent application Ser. No. 16/061,091, filed Jun. 11, 2018, which is a U.S. National Phase application of International Patent Application No. PCT/US2016/029512, filed Apr. 27, 2016, which claims the benefit of U.S. Provisional Patent Application No. 62/266,352, filed Dec. 11, 2015, and U.S. Provisional Patent Application No. 62/279,285, filed Jan. 15, 2016, each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under project number Z01 HG-200318-11 by the National Institutes of Health, National Human Genome Research Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: one 50,902 Byte XML file named "765006.xml," dated Nov. 3, 2022.

BACKGROUND OF THE INVENTION

[0004] Patients with combined methylmalonic acidemia/aciduria and hyperhomocysteinemia/homocystinuria cobalamin C type (cobalamin C deficiency; cblC) are at risk for metabolic decompensation, thromboembolic events, manifest multisystemic disease and require lifelong daily intramuscular injections of cobalamin (vitamin B12) as well as treatment with other expensive medications such as carnitine, folinic acid, and betaine. Because they cannot synthesize adequate levels of methionine, the patients also require constant dietary and metabolic monitoring. Despite medical management, most children with cblC become legally blind by the age of 10 and have other neurological complications such as developmental delay, cognitive impairment, behavioral disorders, psychosis, thromboembolic stokes, and neuropathy.

[0005] There is a need for the development of more effective treatments of cblC, including those that are less burdensome to patients, especially those that treat the neurological and ocular manifestations of the disorder.

BRIEF SUMMARY OF THE INVENTION

[0006] In one embodiment, the present invention provides a synthetic methylmalonic acidemia cblC type with homocystinuria (MMACHC) polynucleotide comprising, consisting essentially of, or consisting of a polynucleotide encoding MMACHC that is codon-optimized for expression in a human. In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of a synthetic MMACHC polynucleotide and a pharmaceutically acceptable carrier.

[0007] In another embodiment, the present invention provides a polypeptide encoded by a synthetic MMACHC polynucleotide. In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of a MMACHC polypeptide and a pharmaceutically acceptable carrier.

[0008] In another embodiment, the present invention provides an expression vector comprising a MMACHC gene sequence under the control of a chicken beta actin (CBA) promoter. In another embodiment, the present invention provides an expression vector comprising a synthetic MMACHC polynucleotide. In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of an expression vector and a pharmaceutically acceptable carrier.

[0009] In another embodiment, the present invention provides a method of treating or preventing at least one condition of methylmalonic acidemia, hyperhomocysteinemia/homocystinuria, cobalamin C type and deficiency of MMACHC, and low levels of MMACHC in a subject, the method comprising, consisting essentially of, or consisting of administering to a subject in need thereof a therapeutically effective amount of (i) a synthetic MMACHC polynucleotide described herein; (ii) a composition described herein; (iii) a polypeptide described herein; or an expression vector described herein; wherein the administration treats the condition in the subject.

[0010] In another embodiment, the present invention provides a method for detecting or tracking exogenous MMACHC in a subject comprising, consisting essentially of, or consisting of: (a) administering to the subject exogenous MMACHC in the form of (i) a synthetic MMACHC polynucleotide described herein, or (ii) an expression vector described herein; (b) obtaining a sample of tissue, biospecimen, or body fluid from the subject; and (c) determining the expression level of the exogenous MMACHC in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIGS. **1**A and **1**B depict the BLASTN alignment between the wild-type MMACHC gene (top sequence; SEQ ID NO: 1) and the codon-optimized allele (bottom sequence; SEQ ID NO: 2). The genes have only 77% identity at the nucleotide level and widespread incomplete alignment. Score: 499 bits (270); Expect: 3e-145; Identities: 656/848 (77%); Gaps: 4/484 (0%); Strand: Plus/Plus.

[0012] FIG. **2** is a line graph showing $Mmachc^{\Delta 3/\Delta 3}$ and $Mmachc^{\Delta 2/\Delta 2}$ mice exhibit reduced survival (**** $p<0.0001$, compared to +/+).

[0013] FIG. **3** is a line graph showing $Mmachc^{\Delta 3/\Delta 3}$ mice exhibit reduced growth. Weight is average weight of pup(s) of the $Mmachc^{\Delta 3/\Delta 3}$ genotype from a given litter compared to heterozygotes and wild-type controls from the same litters on a given day (**** $p<0.0001$).

[0014] FIGS. **4**A-**4**C are dot plots showing $Mmachc^{\Delta 3/\Delta 3}$ mice display a characteristic biochemical phenotype of cblC. FIG. **4**A shows methylmalonic acid levels. FIG. **4**B shows homocysteine levels. FIG. **4**C shows methionine levels. $p<0.05$-0.001 for all metabolites.

[0015] FIG. **5** is a line graph showing hydroxocobalamin (OH-Cbl) treatment improves survival of Mmachc$^{\Delta 3/\Delta 3}$ mice (**** $p<0.001$, log-rank test, compared to Δ3/Δ3).

[0016] FIG. **6** is a line graph showing OH-Cbl treatment improves growth of Mmachc$^{\Delta 3/\Delta 3}$ mice, compared with Δ3/Δ3 (*** $p<0.001$) compared with untreated Mmachc$^{\Delta 3/\Delta 3}$ mice (** $p<0.005$) but does not normalize (*** $p<0.001$) compared to controls.

[0017] FIGS. **7**A-**7**C are dot plots showing OH-Cbl improves biochemical phenotype in Mmachc$^{\Delta 3/\Delta 3}$ mice. FIG. **7**A shows methylmalonic acid levels. FIG. **7**B shows homocysteine levels. FIG. **7**C shows methionine levels. *$p<0.05$, ** $p<0.01$, *** $p<0.001$, "NS" is not significant.

[0018] FIGS. **8**A-**8**D show the expression of various MMACHC constructs. FIG. **8**A shows the codon-optimized (MMACHC cod. op.), MMACHC-HA (MMACHC cod. op.-HA) tagged and MMACHC-3×FLAG-tagged (MMACHC cod.op.-FLAG) protein levels compared to the wild-type mouse Mmachc after transfection of expression constructs into 293T cells and probing the cellular extracts with an anti-MMACHC antibody (AB1). An increased expression level of the codon-optimized alleles compared to the wild-type mouse gene can be easily appreciated. FIG. **8**B shows the codon-optimized MMACHC and tagged alleles compared to the wild-type mouse Mmachc after transfection into 293T cells and probing with a second anti-MMACHC antibody (AB2). An increased expression level of the codon-optimized alleles compared to the wild-type mouse gene can be easily appreciated.

[0019] FIG. **8**C shows the codon-optimized MMACHC and tagged alleles compared to the wild-type mouse Mmachc after transfection into 293T cells and probing with an anti-HA antibody. A single allele containing the HA tag is recognized. FIG. **8**D shows the codon-optimized and tagged MMACHC alleles compared to the wild-type mouse Mmachc after transfection into 293T cells and probing with an anti-FLAG antibody. A single allele containing the FLAG tag is recognized.

[0020] FIGS. **9**A-**9**E are diagrams showing AAV (Adeno-Associated Virus) vector maps. FIG. **9**A shows an AAV vector with the wild-type human MMACHC. FIG. **9**B shows an AAV vector with the wild-type mouse Mmachc. FIG. **9**C shows an AAV vector with a codon-optimized, synthetic human MMACHC. FIG. **9**D shows an AAV vector with a codon-optimized, synthetic human MMACHC tagged with hemagglutinin (HA). FIG. **9**E shows an AAV vector with a codon-optimized, synthetic human MMACHC tagged with 3×FLAG.

[0021] FIG. **10** is a line graph showing neonatal treatment with AAVrh10-CBA-Mmachc or AAV9-CBA-coMACHC improves survival of Mmachc$^{\Delta 3/\Delta 3}$ mice, in accordance with embodiments of the invention (**** $p<0.0001$, compared to untreated Mmachc$^{\Delta 3/\Delta 3}$). The treated mice have about 80% survival at 100 days after a single neonatal injection compared with the untreated mice which do not survive.

[0022] FIG. **11** is a line graph showing that the AAVrh10-CBA-Mmachc treated Mmachc$^{\Delta 3/\Delta 3}$ mice (group 4) have improved weight gain in the first month of life compared with untreated mice (group 5). AAVrh10-CBA-Mmachc treated Mmachc$^{\Delta 3/\Delta 3}$ mice are essentially identical in weight to those that receive OH-Cbl 1-2 times per week (group 3) but remain smaller than wild-type and heterozygous mice.

[0023] FIG. **12** is a line graph showing the weights of the Mmachc$^{\Delta 3/\Delta 3}$ mice treated with OH-Cbl, AAVrh10-CBA-Mmachc or AAV9-CBA-coMMACHC over time. Mmachc$^{\Delta 3/\Delta 3}$ mice treated with a single injection of AAV in the neonatal period are essentially identical in weight to those that receive OH-Cbl 1-2 times per week. All Mmachc$^{\Delta 3/\Delta 3}$ mutant mice remain smaller than wild-type and heterozygous mice.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Cobalamin C deficiency is the most common inborn error of intracellular cobalamin metabolism (Lerner-Ellis et al. Nat. Genet., 38: 93-100 (2006)) and is caused by mutations in MMACHC (GenBank NM_015506.2), a gene responsible for the processing and trafficking of intracellular cobalamin. Mutations in MMACHC impair the activity of two cobalamin-dependent enzymes: methylmalonyl-CoA mutase (MUT) and methionine synthase (MTR). Without wishing to be bound to any theory, MMACHC transports and processes intracellular cobalamin into its two active cofactors, 5'-adenosylcobalamin and methylcobalamin, necessary for the enzymatic reactions of MUT and MTR, respectively. Patients display methylmalonic acidemia, hyperhomocysteinemia, and hypomethionemia and variably manifest intrauterine growth retardation, anemia, heart defects, failure to thrive, white matter disease, neuropathy, neurocognitive impairment, and a progressive maculopathy, pigmentary retinopathy, and retinal degeneration that causes blindness, despite standard of care metabolic therapy (Carrillo-Carrasco et al., J. Inherit. Metab. Dis., 35: 103-14 (2012)).

[0025] Cobalamin deficiency type C (cblC) is often diagnosed based on newborn screening. While the true prevalence of the disorders of intracellular cobalamin metabolism is unknown, the historical incidence of cblC has been estimated at 1:200,000 births with about 400 cases reported in the literature; recently, data from newborn screening suggested a higher incidence closer to 1:100,000 in New York state and 1:60,000 in California, where an incidence of 1:37,000 was estimated in the Hispanic population. In one study of a Chinese population in Shangong province, it was claimed that 1:3920 births were affected (Han et al., Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic acidemia and homocysteinemia (cblC type) in Shandong province, China, Brain Dev., pii: 50387-7604(15)00228-4 (2015)).

[0026] Previous efforts to develop a viable animal model of cblC have proven unsuccessful. A knockout mouse generated from an Mmachc gene trap resulted in embryonic arrest by day E3.5 (Moreno-Garcia et al., Mol. Genet. Metab., 112: 198-204 (2014)). As shown in the Examples below, viable mouse models (Mmachc$^{\Delta 2/\Delta 2}$ and Mmachc$^{\Delta 3/\Delta 3}$) were developed and are described herein. The mouse models exhibit the phenotypic and biochemical features of patients with cblC. When treated using a conventional treatment (hydroxocobalamin), Mmachc$^{\Delta 3/\Delta 3}$ mice displayed improvement in disease characteristics, and Mmachc$^{\Delta 3/\Delta 3}$ mice have been used in developing treatments for patients with cblC, as described herein.

[0027] In one embodiment, the present invention provides a synthetic MMACHC polynucleotide comprising, consisting essentially of, or consisting of a polynucleotide encoding MMACHC that is codon-optimized for expression in a human. FIG. **1** shows a comparison of SEQ ID NO: 1 (wild-type MMACHC) with SEQ ID NO: 2 (codon-opti-

mized MMACHC). In another embodiment, the polynucleotide encoding MMACHC comprises, consists essentially of, or consists of the sequence of SEQ ID NO: 2. In another embodiment, the polynucleotide encoding MMACHC has at least about 90% sequence homology with SEQ ID NO: 2. In another embodiment, the polynucleotide encoding MMACHC has at least about 95% sequence homology with SEQ ID NO: 2. The codon-optimized MMACHC gene or homologous gene can be associated with any suitable stop codon, for example the stop codon of TAA or TGA or together as two contiguous codons (TAATGA).

[0028] A polynucleotide is a nucleic acid. "Nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In some embodiments, the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions, such as with codon optimization.

[0029] The nucleic acids may be recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by Joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be in vitro replication or in vivo replication.

[0030] A recombinant nucleic acid may be one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques, which are well known in the art. The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxy acetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids can be purchased from companies.

[0031] Contemplated herein are any isolated or purified nucleotide sequence described herein. Alternatively, the nucleotide sequence can comprise a nucleotide sequence which is degenerate to any of the sequences or a combination of degenerate sequences or which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

[0032] The nucleotide sequence that hybridizes under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions that would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70° C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0033] The terms "complementary" and "complementarity" refer to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base-paring or other non-traditional types of pairing. The degree of complementarity between two nucleic acid sequences, i.e., the homology, can be indicated by the percentage of nucleotides in a nucleic acid sequence which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 50%, 60%, 70%, 80%, 90%, and 100% complementary). Two nucleic acid sequences are "perfectly complementary" if all the contiguous nucleotides of a nucleic acid sequence will hydrogen bond with the same number of contiguous nucleotides in a second nucleic acid sequence. Two nucleic acid sequences are "substantially complementary" if the degree of complementarity between the two nucleic acid sequences is at least 60% (e.g., 65%, 70%, 75%, 80%, 85%, 90%, 95%. 97%, 98%, 99%, or 100%) over a region of at least 8 nucleotides (e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides).

[0034] A codon-optimized polynucleotide is a polynucleotide, for example a naturally-occurring polynucleotide, that has been altered to improve expression in an organism, for example, a human or a cell line derived from a human.

[0035] In another embodiment, the polynucleotide further comprises a tag, wherein the tag can be an epitope tag. In another embodiment, the polynucleotide further comprises a polynucleotide encoding at least one of a hemagglutinin tag and a 3×FLAG tag. A tag facilitates detection, and epitope tags are protein regions that can be identified using immunoassay techniques. For example, an epitope tag can be recognized by an antibody or a binding portion thereof (e.g., scFv). In another embodiment, the polynucleotide encoding the hemagglutinin tag comprises, consists essentially of, or consists of the sequence of SEQ ID NO: 3. In another embodiment, the polynucleotide encoding the hemagglutinin tag has at least about 90% sequence homology with SEQ ID NO: 3. In another embodiment, the polynucleotide encoding the hemagglutinin tag has at least about 95% sequence homology with SEQ ID NO: 3. In another embodiment, the polynucleotide encoding the 3×FLAG tag comprises, consists essentially of, or consists of the sequence of SEQ ID NO: 4. In another embodiment, the polynucleotide encoding the 3×FLAG tag has at least about 90% sequence homology with SEQ ID NO: 4. In another embodiment, the polynucleotide encoding the 3×FLAG tag has at least about 95% sequence homology with SEQ ID NO: 4. In yet another embodiment, the tag can include others recognized by practitioners of the art, including His, c-myc, GST, Protein A, CD, Strep-tag, MBP, CBD, S-tag, Avitag, CBP, TAP, SF-TAP or others that would allow facile detection, purification, assay and biodistribution of the tag affixed to a codon-optimized MMACHC protein or nucleic acid.

[0036] In another embodiment, the present invention provides a polypeptide encoded by a synthetic MAACHC polynucleotide. The polypeptide can be altered so long as the polypeptide has substantially the same activity as wild-type MMACHC and can include a functional portion of MMACHC. The term "functional portion" refers to any part or fragment of MMACHC, which part or fragment retains the biological activity of MMACHC. Functional portions encompass, for example, those parts of MMACHC that retain the ability of processing and trafficking intracellular cobalamin. In reference to MMACHC, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of MMACHC.

[0037] The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of MMACHC. Desirably, the additional amino acids do not interfere with the biological function of the functional portion. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of MMACHC.

[0038] Contemplated herein are functional variants of MMACHC. The term "functional variant" as used herein refers to a polypeptide or protein having substantial or significant sequence identity or similarity to MMACHC, which functional variant substantially retains the biological activity of MMACHC. Functional variants encompass, for example, those variants of MMACHC that retain the ability of processing and trafficking intracellular cobalamin to a similar extent, the same extent, or to a higher extent, as MMACHC. In reference to MMACHC, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to MMACHC.

[0039] A functional variant can, for example, comprise the amino acid sequence of MMACHC with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of MMACHC with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to MMACHC.

[0040] Amino acid substitutions are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic amino acid substituted for another acidic amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Val, etc.), a basic amino acid substituted for another basic amino acid (Lys, Arg, etc.), an amino acid with a polar side chain substituted for another amino acid with a polar side chain (Asn, Cys, Gln, Ser, Thr, Tyr, etc.), etc.

[0041] The MMACHC polypeptide can consist essentially of the specified amino acid sequence or sequences described herein, such that other components e.g., other amino acids, do not materially change the biological activity of the functional variant.

[0042] The MMACHC polypeptide (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the MMACHC (or functional portions or functional variants thereof) retain their biological activity, e.g., of processing and trafficking intracellular cobalamin. For example, the polypeptide can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length. In this regard, the polypeptides also include oligopeptides.

[0043] The MMACHC polypeptide (including functional portions and functional variants) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0044] The MMACHC polypeptide (including functional portions and functional variants) can be glycosylated, ami-

dated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

[0045] The MMACHC polypeptide (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of de novo synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Pat. No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, N Y, 1994.

[0046] Further, MMACHC (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the MMACHC (including functional portions and functional variants thereof) can be commercially synthesized by companies. In this respect, the MMACHC can be synthetic, recombinant, isolated, and/or purified.

[0047] The term "isolated" as used herein means having been removed from its natural environment. The term "purified" or "isolated" does not require absolute purity or isolation; rather, it is intended as a relative term. Thus, for example, a purified (or isolated) protein preparation is one in which the protein is more pure than the protein in its natural environment within a cell. Such proteins may be produced, for example, by standard purification techniques, or by recombinant expression. In some embodiments, a preparation of a protein is purified such that the protein represents at least about 50%, for example at least about 70%, of the total protein content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

[0048] An embodiment provides recombinant expression vectors comprising any of the nucleic acids described herein. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0049] In an embodiment, the recombinant expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from bacterial expression systems such as PET or one that can direct expression in yeast such as pYES or even used to make a mammalian cell line for overproduction of MMACHC in such cells as Chinese hamster ovary or others know to practitioners of the art.

[0050] In another embodiment, the codon-optimized MMACHC gene and tagged alleles can be used, in combination with genome editing and homologous recombination, to create cell lines that over express MMACHC and/or tagged alleles from a specific endogenous genomic location, such as in the albumin gene, or from a safe-harbor. Inducible and regulatable control of the recombinant MMACHC alleles is also envisioned.

[0051] The terms "transfection," "transformation," or "transduction," as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, e.g., Murray E. J. (ed.), Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature,* 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.,* 7: 2031-2034 (1987)).

[0052] In an embodiment, the recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., supra, and Ausubel et al., supra. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColE1, 2μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0053] The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) or tissue into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based.

[0054] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0055] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the MMACHC nucleotide sequence (including functional portions and functional variants thereof), or to the nucleotide

sequence which is complementary to or which hybridizes to the nucleotide sequence encoding MMACHC. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus. In another embodiment, the present invention provides an expression vector comprising a MMACHC gene sequence under the control of a chicken beta actin (CBA) promoter. In another embodiment, the present invention provides an expression vector comprising a synthetic MMACHC polynucleotide. In another embodiment, the synthetic MMACHC polynucleotide is under the control of a chicken beta actin (CBA) promoter.

[0056] In addition to the nucleic acid sequence encoding the MMACHC, the vector preferably comprises expression control sequences, such as promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the nucleic acid sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990). The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0057] The recombinant expression vector may be a viral vector, e.g., a retroviral vector. In another embodiment, the expression vector is a viral vector. In another embodiment, the vector is an adenovirus, or a helper-dependent adenovirus. In another embodiment, the vector is a herpes viral vector. In yet another embodiment, the vector is an integrating vector, such as a lentiviral vector.

[0058] In another embodiment, the viral vector is a single-stranded adeno-associated viral (AAV) vector. The AAVs can be derived from serotypes well-known to practitioners of the art, including AAV 1, 2, 3, 4, 5, 6, 8, 9, rh8, rh10, rh33, and rh34. Chimeras between these serotypes and point mutations of said serotypes are contemplated, such as tyrosine mutants of serotypes 2, 5, 6, 8 and 9, as are mutants in surface exposed tyrosine (Y) and threonine (T) residues on the capsids (Bogner et al., PLoS One, 10(6):e0128759 (2015); Kay et al., PLoS One, 8(4):e62097 (2013), erratum in: PLoS One, 8(9) (2013); Qiao et al., Hum. Gene Ther. Methods, 23(1):29-37 (2012); Ryalsv et. al.., Mol Vis., 17:1090-102 (2011); Markusic et al., Mol. Ther., 18(12): 2048-56 (2010); Qiao et al., Hum. Gene Ther., 21(10): 1343-8 (2010); Petrs-Silva et al., Mol. Ther., 17(3):463-71 (2009); each of which are individually incorporated by reference herein in its entirety). Also considered are capsids derived by in vitro evolution and selection, such as 7m8 (Dalkara et al., Sci. Transl. Med., 5(189):189ra76 (2013), incorporated by reference herein in its entirety) and those derived by phylogenetic reconstruction such as Anc80 and relatives (Santiago-Ortiz et al., Gene Ther., 22(12):934-46 (2015), incorporated by reference herein in its entirety). The vector can be, for example, Anc80 and others as described in Zinn et al., Cell Rep., 12(6):1056-1068 (2015), incorporated by reference herein in its entirety.

[0059] In another embodiment, the viral vector is constructed in the self-complimentary (sc) configuration as is well known to practitioners of the art. scAAVs designed to express the codon-optimized MMACHC gene or tagged alleles may have increased potency and can be encapsidated with any of the aforementioned serotypes.

[0060] The AAVs expressing MMACHC and variants can be purified after transfection methods using 293 cells of an equivalent, produced in insect cells or using herpes-based systems. Such methods are well known to practitioners of the art.

[0061] Using an AAV expressing MMACHC or one configured to correct an MMACHC patient cell line, hematopoietic stem and progenitor cells can be transduced with an AAV6 donor and homologous recombination can be used to correct the mutant allele using genome editing (Wang et al., Nat. Biotechnol., 33(12):1256-1263 (2015), incorporated by reference herein in its entirety). This can be accomplished with mRNA expressing ZFNs, TALENS or the CAS-CRISPR system. The corrected or transduced MMACHC cells can then be used as a source of cellular therapy.

[0062] In another embodiment, an AAV vector can be designed to enable promoterless correction, either at a safe harbor location in the genome or into predetermined cellular target gene, ex vivo or in vivo (Barzel et al., Nature, 517(7534):360-364 (2015), incorporated by reference herein in its entirety).

[0063] The AAV may be selected such that there is improvement of infection of a target tissue. In another embodiment, the AAV is pseudotyped with at least one of rh10, type 9, or type 8 capsid. Further examples include the AAVs of U.S. Patent Publication No. 2014/0364338, incorporated by reference herein in its entirety, which have altered capsid proteins for greater infectivity of retinal cells.

[0064] In another embodiment, the present invention provides an expression vector comprising, consisting essentially of, or consisting of any one of SEQ ID NOS: 5-9. In another embodiment, the present invention provides an expression vector comprising, consisting essentially of, or consisting of at least about 90% sequence homology with any one of SEQ ID NOS: 5-9. In another embodiment, the present invention provides an expression vector comprising, consisting essentially of, or consisting of at least about 95% sequence homology with any one of SEQ ID NOS: 5-9.

[0065] In another embodiment, the present invention provides a method of treating or preventing at least one condition of methylmalonic acidemia, homocystinuria, cobalamin C type and deficiency of MMACHC, and low levels of MMACHC in a subject, the method comprising, consisting essentially of, or consisting of administering to a subject in need thereof a therapeutically effective amount of (i) a synthetic MMACHC polynucleotide described herein; (ii) a composition described herein; (iii) a polypeptide described herein; or an expression vector described herein; wherein the administration treats the condition in the subject.

[0066] In another embodiment, the present invention provides a method of treating or preventing a disorder associated with MMACHC deficiency such as congenital heart defects (CHD), neural tube defects (NTD), combined methylmalonic acidemia and homocystinuria X type (cblX), HCFC1 spectrum defects, hyperhomocystinuria, and vita-

min B12 deficiency (including recalcitrant vitamin B12 deficiency) in a subject, the method comprising, consisting essentially of, or consisting of administering to a subject in need thereof a therapeutically effective amount of (i) a synthetic MMACHC polynucleotide described herein; (ii) a composition described herein; (iii) a polypeptide described herein; or an expression vector described herein; wherein the administration treats the condition in the subject. As an example, patients with cblX deficiency caused by HCFC1 mutations are also deficient in the expression of MMACHC and suffer from combined methylmalonic acidemia and hyperhomocystinemia (Yu et al., Am. J. Hum. Gen., 93: 506-514 (2013), incorporated by reference herein in its entirety) and would benefit from increased MMACHC.

[0067] In another embodiment, the subject has vision loss and the administration is to the eye(s) of the subject. Most patients with cblC develop a bulls' eye maculopathy during infancy despite early identification by newborn screening and treatment with hydroxocobalamin and other cofactors. In early childhood, retinal degeneration progresses and optic nerve atrophy develops resulting in progressive vision loss until most children are legally blind by the end of the first decade of life. Prior to the present invention, there was no treatment for the eye disease of cobalamin C deficiency or deficiency of MMACHC, or the vision loss that accompanies it. A specific form of AAV mediated ocular gene therapy is envisioned using the invention described herein. The AAV can be delivered by subretinal injection, intravitreal injection, and/or retinal artery or vein injection.

[0068] In another embodiment, the present invention provides a method for detecting or tracking exogenous MMACHC in a subject comprising, consisting essentially of, or consisting of: (a) administering to the subject exogenous MMACHC in the form of (i) a synthetic MMACHC polynucleotide described herein, or (ii) an expression vector described herein; (b) obtaining a sample of tissue, biospecimen, or body fluid from the subject; and (c) determining the expression level of the exogenous MMACHC in the sample. Determining the expression level can be achieved by, for example, detecting or tracking RNA or protein levels of MMACHC. Detecting or tracking protein levels can involve, for example, detecting an expression tag associated with the MMACHC (e.g., using Western blot techniques), as described herein.

[0069] Further, the activity of MMACHC and its distribution can be detected or tracked using nucleic acid probes and nucleic acid detection methods. Because the codon-optimized MMACHC and tagged alleles share only 77% or less identity with wild-type at the nucleotide level, a facile detection using nucleic acid methods is envisioned. This includes qPCR, droplet PCR, RT-PCR as well as hybridization and polymerase amplification methods. In another embodiment, mass spectrometry and MALDI-TOF methods can be used to detect the codon-optimized nucleic acid in solution, tissues samples, biospecimens, and body fluids.

[0070] A biospecimen may include any tissue or cells, including white and red blood cells, saliva and salivary DNA, cell shed in the urine or feces, or any other organ that requires more invasive assessment by biopsy, such as liver, eye, retina, kidney, bone or skeletal muscle. A body fluid might include urine, plasma, serum, cerebrospinal fluid, aqueous humor, or feces.

[0071] In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of a synthetic MMACHC polynucleotide and a pharmaceutically acceptable carrier. In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of a MMACHC polypeptide and a pharmaceutically acceptable carrier. In another embodiment, the present invention provides a composition comprising, consisting essentially of, or consisting of an expression vector and a pharmaceutically acceptable carrier.

[0072] In certain embodiments, pharmaceutical composition are contemplated, comprising any of the nucleic acids, polypeptides, proteins, functional portions, functional variants, and expression vectors described herein and a pharmaceutically acceptable carrier. The pharmaceutical compositions can comprise more than one material, e.g., a polypeptide and a nucleic acid. Alternatively, the pharmaceutical composition can comprise a combination with other pharmaceutically active agents or drugs.

[0073] With respect to pharmaceutical compositions, the pharmaceutically acceptable carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active agent(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0074] Methods for preparing administrable (e.g., parenterally administrable) compositions are known or apparent to those skilled in the art and are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0075] The following formulations for oral, aerosol, parenteral (e.g., subcutaneous, intravenous, portal, intraarterial, intramuscular, intradermal, interperitoneal, and intrathecal), and rectal, and vaginal administration are merely exemplary and are in no way limiting. More than one route can be used for administration, and in certain instances, a particular route can provide a more immediate and more effective response than another route. The delivery of codon-optimized MMACHC via subretinal, intravitreal, transcranial, or epidural routes can also be used.

[0076] Formulations suitable for oral administration can comprise or consist of (a) liquid solutions, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard or softshelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and

other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can include a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the material in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

[0077] Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Administration can be in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, poly(ethylenegly col) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0078] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0079] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0080] In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0081] Injectable formulations are contemplated. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., Pharmaceutics and Pharmacy Practice, J.B. Lippincott Company, Philadelphia, Pa., Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986)).

[0082] Topical formulations, including those that are useful for transdermal drug release, are well known to those of skill in the art and are suitable for application to skin.

[0083] The gene and alleles can also be formulated as nucleic acids and encapsulated into lipid nanoparticles, or prepared as peptide nucleic acids and delivered in this fashion.

[0084] An "effective amount" or "an amount effective to treat" or "therapeutically effective amount" refers to a dose that is adequate to prevent or treat cobalamin C disease or MMACHC deficiency in an individual. Amounts effective for a therapeutic or prophylactic use will depend on, for example, the stage and severity of the disease or disorder being treated, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the active selected, method of administration, timing and frequency of administration, the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular active, and the desired physiological effect. For example, the dosing may range from a single systemic AAV injection at a dose of 2.5×10^{9} genome copies/kilogram to a higher dose, on the order of $1\times10^{10\text{-}12}$ genome copies per eye, delivered by intravitreous or subretinal injection.

[0085] Additionally, the use of MMACHC, as a treatment for other forms of hyperhomocysteinemia, is encompassed herein.

[0086] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of cblC in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of cblC being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[0087] The subject can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the

order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0088] It shall be noted that the preceding are merely examples of embodiments. Other exemplary embodiments are apparent from the entirety of the description herein. It will also be understood by one of ordinary skill in the art that each of these embodiments may be used in various combinations with the other embodiments provided herein.

[0089] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

Example 1

[0090] This example demonstrates the development of mouse models of human cblC.

[0091] To create a viable animal model of cblC deficiency, TALEN-mediated genome editing (Transposagen Biopharmaceuticals, Lexington, Ky., USA) was used to edit exon 2 of Mmachc in mice, near the location of the common mutation seen in humans—c.271dupA p.R91KfsX14. This technique is known to practitioners of the art (Qiu et al., Nucleic Acids Res., 41:e120 (2013), incorporated by reference herein in its entirety). Eleven founder mice harboring 10 different alleles were generated. Two mutations were further investigated: an early frameshift null allele [cDNA mutation c.165_166delAC giving protein mutation p.P56CfsX4 (Δ2)] and another [cDNA mutation c.162_164delCAC giving protein mutation p.S54_T55delinsR (Δ3)] that results in a deletion-insertion predicted to produce an intact but mutant enzyme. Founder mice harboring mutant alleles were bred to make stable transmitting lines, which were then intercrossed to produce homozygote affected animals with Mmachc deficiency.

[0092] At birth, an expected 1:2:1 Mendelian segregation was observed for the $Mmachc^{\Delta 3}$ allele (n=30 litters, 218 mice, χ^2 $p>0.1$) but not for $Mmachc^{\Delta 2}$ (n=19 litters, 134 mice, χ^2 $p<0.001$), in which the proportion of $Mmachc^{\Delta 2/\Delta 2}$ mice was decreased, suggesting partial embryonic lethality caused by this mutation (Table 1).

TABLE 1

Mmachc parental genotype		Offspring Mmachc genotype distribution			Number			
Dam	Sire	+/+	+/−	−/−	of litters	χ^2	df	p-value
+/Δ3	+/Δ3	54	120	44	30 (n = 218)	3.13	2	p >0.1
+/Δ2	+/Δ2	39	81	14	19 (n = 134)	15.18	2	p <0.001

All mice have FVB/N C57/B6 mixed background

[0093] $Mmachc^{\Delta 2/\Delta 2}$ and $Mmachc^{\Delta 3/\Delta 3}$ mice were growth retarded and displayed decreased survival with 100% lethality by 32 days (FIGS. **2** and **3**). Survival of heterozygote mice does not differ significantly from wild-type mice for either allele. The survival curves for Mmachc and Mmachc mice do not differ significantly.

[0094] Compared with wild-type controls (n=7), $Mmachc^{\Delta 2/\Delta 2}$ (n=4) and $Mmachc^{\Delta 3/\Delta 3}$ (n=6) mutants displayed significantly elevated plasma methylmalonic acid (FIG. **4**A), homocysteine (FIG. **4**B), and decreased methionine (FIG. **4**C). Also, $Mmachc^{\Delta 3/\Delta 3}$ mice display hepatic lipidosis and eye pathology similar to human cblC, including thinner outer nuclear layer with fewer nuclei and reduction in photoreceptor outer segments.

[0095] These mouse models represent viable mammalian models of cblC deficiency and recapitulate the phenotypic and biochemical features of the human disorder. The models presented with cblC-related biochemical perturbations (methylmalonic acidemia, hyperhomocysteinemia, and hypomethioninemia), exactly as present in the human patient population. In addition, both mutants showed hypopigmentation.

Example 2

[0096] This example demonstrates treatment of cblC in $Mmachc^{\Delta 3/\Delta 3}$ mice using hydroxocobalamin (OH-Cbl).

[0097] Mice received prenatal treatment via maternal injections of 25-50 μg two times per week and postnatal treatment via injection of the same dose of 25-50 μg two to three times per week.

[0098] $Mmachc^{\Delta 3/\Delta 3}$ mice treated with OH-Cbl display significantly increased survival compared to non-treated $Mmachc^{\Delta 3/\Delta 3}$ mice (FIG. **5**). Survival of $Mmachc^{\Delta 3/\Delta 3}$ mice treated with OH-Cbl does not differ significantly from treated $Mmachc^{+/+}$ mice.

[0099] $Mmachc^{\Delta 3/\Delta 3}$ mice treated with OH-Cbl display increased growth compared to non-treated $Mmachc^{\Delta 3/\Delta 3}$ mice (FIG. **6**).

[0100] $Mmachc^{\Delta 3/\Delta 3}$ mice treated with OH-Cbl also display improvement in biochemical phenotype (FIGS. **7**A-**7**C). Methylmalonic acid and methionine were found to be more dependent on diet, where a larger sample size is needed.

[0101] Also, it was found that OH-Cbl treatment reverses hypopigmentation in $Mmachc^{\Delta 3/\Delta 3}$ mice.

Example 3

[0102] This example demonstrates the development of improved expression of MMACHC by codon optimization.

[0103] Adeno-associated viruses (AAVs) were constructed that were designed to broadly express either (i) the wild-type human MMACHC (SEQ ID NO: 1, and having stop codon TAA); (ii) the wild-type mouse Mmachc (SEQ ID NO: 10, and having stop codon TAA); (iii) a codon-optimized, synthetic human MMACHC (SEQ ID NO: 2, and having stop codons TAA and TGA; (iv) a codon-optimized, synthetic human MMACHC tagged with hemagglutinin (HA) (SEQ ID NO: 11, and having stop codons TAA and TGA; or (v) a codon-optimized, synthetic human MMACHC tagged with 3×FLAG (SEQ ID NO: 12, and having stop codons TAA and TGA. All constructs were under the control of the enhanced chicken beta actin promoter (CBA). Each plasmid at 2.5 micrograms was transfected into 293T cells, the cells were harvested and immunoreactive MMACHC was detected with two distinct MMACHC antibodies by Western blotting. FIGS. **8**A and **8**B show increased expression of the codon-optimized alleles compared to the wild-type Mmachc. FIG. **8**C shows the specificity of the HA tag, and FIG. **8**D shows the specificity of the FLAG tag.

Example 4

[0104] This example demonstrates the development of gene therapies for cblC.

[0105] Adeno-associated viruses (AAVs) were constructed that were designed to broadly express either (i) the wild-type human MMACHC (SEQ ID NO: 1, and having stop codon TAA); (ii) the wild-type mouse Mmachc (SEQ ID NO: 10, and having stop codon TAA, in rh10 serotype; vector termed AAVrh10-CBA-Mmachc); (iii) a codon-optimized, synthetic human MAACHC (SEQ ID NO: 2, and having stop codons TAA and TGA, in AAV9 serotype; vector termed AAV9-CBA-coMMACHC); (iv) a codon-optimized, synthetic human MMACHC tagged with hemagglutinin (HA) (SEQ ID NO: 11, and having stop codons TAA and TGA; or (v) a codon-optimized, synthetic human MMACHC tagged with 3×FLAG (SEQ ID NO: 12, and having stop codons TAA and TGA. All constructs were under the control of the enhanced chicken beta actin promoter (CBA). The recombinant AAVs were pseudotyped with an rh10 or type 9 capsid and delivered to mice in the early natal period by direct liver injection. The AAV sequences corresponding to (i)-(v) are at SEQ ID NOS: 5-9, respectively, and are diagrammatically shown at FIGS. **9**A-**9**E, respectively.

[0106] AAVs based on the vectors (ii) and (iii) above were produced in vitro, and mice received a single intrahepatic injection of 1×10^{11} genome copies of AAV (ii) or (iii) per mouse at postnatal day 0-2 using previously described methods (Chandler et al., Mol. Ther., 18(1):11-16 (2010), incorporated by reference herein in its entirety).

[0107] After one month, when 100% of untreated mice had perished, greater than 75% of the treated $Mmachc^{\Delta3/\Delta3}$ mice were alive and the long term survival (6 months) was greater than 50% (FIG. **10**). Of note, the mice treated with the AAV 9 vector that expresses the codon-optimized MMACHC, 100% were alive greater than 60 days, with improved clinical appearance. $Mmachc^{\Delta3/\Delta3}$ mice treated with AAV vectors (AAVrh10 n=11, AAV9 n=5) displayed dramatically improved clinical appearance with improved growth ($p=0.0568$), and increased survival ($p<0.0001$ for both vectors), with the oldest treated mutants living beyond 9 months.

[0108] The gene therapies appear more effective than OH-cbl injections in terms of survival. FIG. **10** also shows the OH-Cbl treatment of $Mmachc^{\Delta3/\Delta3}$ mice.

[0109] Gene therapy (GT) treated $Mmachc^{\Delta3/\Delta3}$ mice remain small (FIG. **11**), and hypopigmentation in a gene therapy treated mouse increases over time, but achieve the same growth parameters as those treated by tri-weekly OH-cbl injections.

[0110] Mmachc mice were treated with OHcbl 1-2 times per week or a single injection of AAV9-CBA-coMMACHC or AAVrh10-CBA-Mmachc and weighed monthly (FIG. **12**). For this study, the animals were maintained on a high fat, fruit, and enterocal enriched diet because of previous work suggesting that such a diet may aid in the survival of animals with isolated methylmalonic acidemia (Chandler et al, FASEB J, 23:1252-61 (2009)). The prolonged intake of such a high fat, carbohydrate enriched diet can cause unaffected or Mmachc carriers to develop increased weight gain and obesity, which explains why the control and heterozygous mice (Group 1, FIG. **12**) in this specific study are much larger than the treated mutants or other controls after 7 months ingesting this diet, as compared to the 40 day old mice control mice presented in FIG. **11** (Group 1).

[0111] A small number of mice treated with gene therapy were sacrificed for ocular studies. The mice display thinning of the outer segments and a retinopathy that is similar to what has been described in humans. Specifically, there was a loss of inner and outer photoreceptors and thinning of the outer nuclear layer, with a reduced number of cells in the outer nuclear layer and shortening of photoreceptor outer segments. Hence, gene therapy was used to create cblC mice that then survive long enough to display the eye disease, which can then be further treated by the invention described herein.

[0112] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0113] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Also, everywhere "comprising" (or its equivalent) is recited, the "comprising" is considered to incorporate "consisting essentially of" and "consisting of." Thus, an embodiment "comprising" (an) element(s) supports embodiments "consisting essentially of" and "consisting of" the recited element(s). Everywhere "consisting essentially of" is recited is considered to incorporate "consisting of" Thus, an embodiment "consisting essentially of" (an) element(s) supports embodiments "consisting of" the recited element(s). "Consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claim.

[0114] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0115] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

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1.-**13**. (canceled)

13. A polypeptide encoded by a synthetic methylmalonic aciduria cblC type with homocystinuria (MMACHC) polynucleotide comprising a polynucleotide encoding MMACHC that is codon-optimized for expression in a human.

14. (canceled)

15. An expression vector comprising a MMACHC gene sequence under the control of a chicken beta actin (CBA) promoter.

16.-**17**. (canceled)

18. The expression vector of claim **15**, wherein the expression vector is a viral vector.

19. The expression vector of claim **18**, wherein the viral vector is an adeno-associated viral (AAV) vector.

20. The expression vector of claim **19**, wherein the AAV is pseudotyped with at least one of rh10, type 9, type 8, and 7m8 capsid.

21.-**24**. (canceled)

25. A method of treating or preventing at least one condition of methylmalonic acidemia, hyperhomocysteinemia, homocystinura, cobalamin C type and deficiency of MMACHC, and low levels of MMACHC in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a synthetic methylmalonic aciduria cblC type with homocystinuria (MMACHC) polynucleotide comprising a polynucleotide encoding MMACHC that is codon-optimized for expression in a human, wherein the administration treats the condition in the subject.

26.-**29**. (canceled)

30. The method of claim **25**, wherein the condition includes vision loss.

31. A method of treating or preventing at least one condition of congenital heart defects (CHD), neural tube defects (NTD), combined methylmalonic acidemia and homocystinuria X type (cblX), HCFC1 spectrum defects, hyperhomocystinuria, and vitamin B12 deficiency in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a synthetic methylmalonic aciduria cblC type with homocystinuria (MMACHC) polynucleotide comprising a polynucleotide encoding MMACHC that is codon-optimized for expression in a human, wherein the administration treats the condition in the subject.

32.-**37**. (canceled)

38. A method of detecting or tracking exogenous MMACHC in a subject comprising (a) administering to the subject exogenous MMACHC in the form of a synthetic methylmalonic aciduria cblC type with homocystinuria (MMACHC) polynucleotide comprising a polynucleotide encoding MMACHC that is codon-optimized for expression in a human; (b) obtaining a sample of tissue, biospecimen, or body fluid from the subject; and (c) determining the expression level of the exogenous MAACHC in the sample.

39. The method of claim **38**, wherein the expression vector is Anc80.

40. The method of claim **38**, wherein the expression vector is an AAV vector pseudotyped with at least one of rh10, type 9, type 8, and 7m8 capsid.

* * * * *