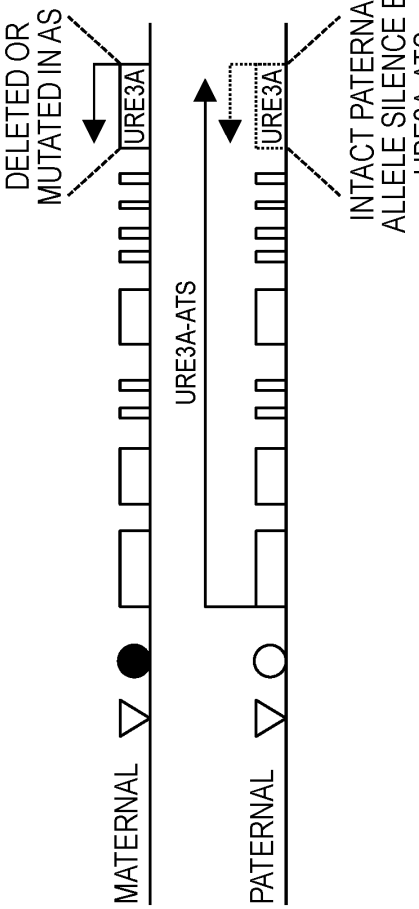


FIG. 1

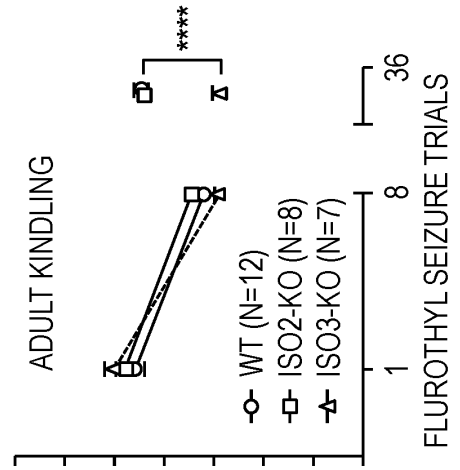


FIG. 2B

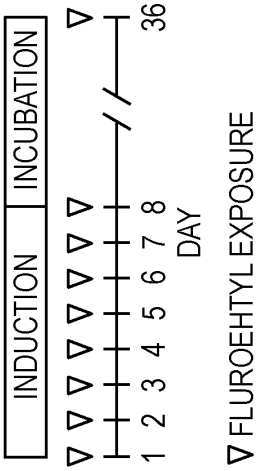


FIG. 2A

HUMAN UBE3A ISOFORMS

SHORT – 1 MKRAAAKHLIERYYHQLTEGCGNEACTNEFCASC

LONG – 2 MEKLHQCYWKSGEPQSDDIEASRMKRAAAKHLIERYYHQLTEGCGNEACTNEFCASC

LONG – 3 MATACKRSGEPQSDDIEASRMKRAAAKHLIERYYHQLTEGCGNEACTNEFCASC

MOUSE UBE3A ISOFORMS

LONG – 2 MATACKRSPGESQSEDIEASRMKRAAAKHLIERYYHQLTEGCGNEACTNEFCASC

SHORT – 3 MKRAAAKHLIERYYHQLTEGCGNEACTNEFCASC

FIG. 3A

WEAK KOZAK

STRONG KOZAK

DNA

GAATTCCGGATTTTTTATGGCCACAGCTTGTAAAGATCAGGAGAACCTCAGTCTGACGACATTGAAGCTAGCAGGATGAAG

PROTEIN

MATACKRSGEPQSDDIEASRMKRAAAKHLIERYYHQLTEGCGNEACTNEFCASCPTFLRMDNNAAAIKALELYKINAKLCDP

HPSKKGASSAYLENSKGAPNNSCSEIKMNKKGARIDFKDVTYLTEEKVYEILELCRERDYSPLIRVIGRVFSSAEALVQSF

RKVKQHTKEELKSLQAKDEDKDEDEKEKAACSAAAMEEDSEASSSRIGDSSQGDNNLQKLGPDDVSVDIDAIRRVYTRLLSN

EKIETAFLNALVYLSPNVECDLTYHNVYSRDPNYLNLFIIVMENRNLHSPEYLEMALPLFCKAMSKLPLAAQGKLIRLWSKY

NADQIRRMMETFQQLITYKVISNEFNSRNLVNDDDAIVAASKCLKMVYYANVVGGEVDTNHNEEDDEEPIPESSELTLQELL

GEERRNKKGPRVDPLETELGVKTLDCRKPLIPFEEFINEPLNEVLEMDKDYTFFKVETENKFSFMTCPFILNAVTKNLGLYY

DNRIRMYSERRITVLYSLVQGQQLNPYLRLKVRRDHIIDDALVRLEMIAMENPADLKKQLYVEFEGEQGVDEGGVSKEFFQL

VVEEIFNPDIGMFTYDESTKLFWFNPSSFETEGQFTLIGIVLGLAIYNNCILDVHFPMVVYRKLMGKKGTFRDLGDSHPVLY

QSLKDLLEYEGNVEDDMMITFQISQTDLFGNPMMYDLKENGDKIPITNENRKEFVNLYSDYILNKSVEKQFKAFRRGFHMVT

NESPLKYLFRPEEIELLICGSRNLDFQALEETTEYDGGYTRDSVLIREFWEIVHSFTDEQKRLFLQFTTGTDRAPVGGLGKL

KMIIAKNGPDTERLPTSHTCFNVLLLPEYSSKEKLKERLLKAITYAKGFGML

FIG. 3B

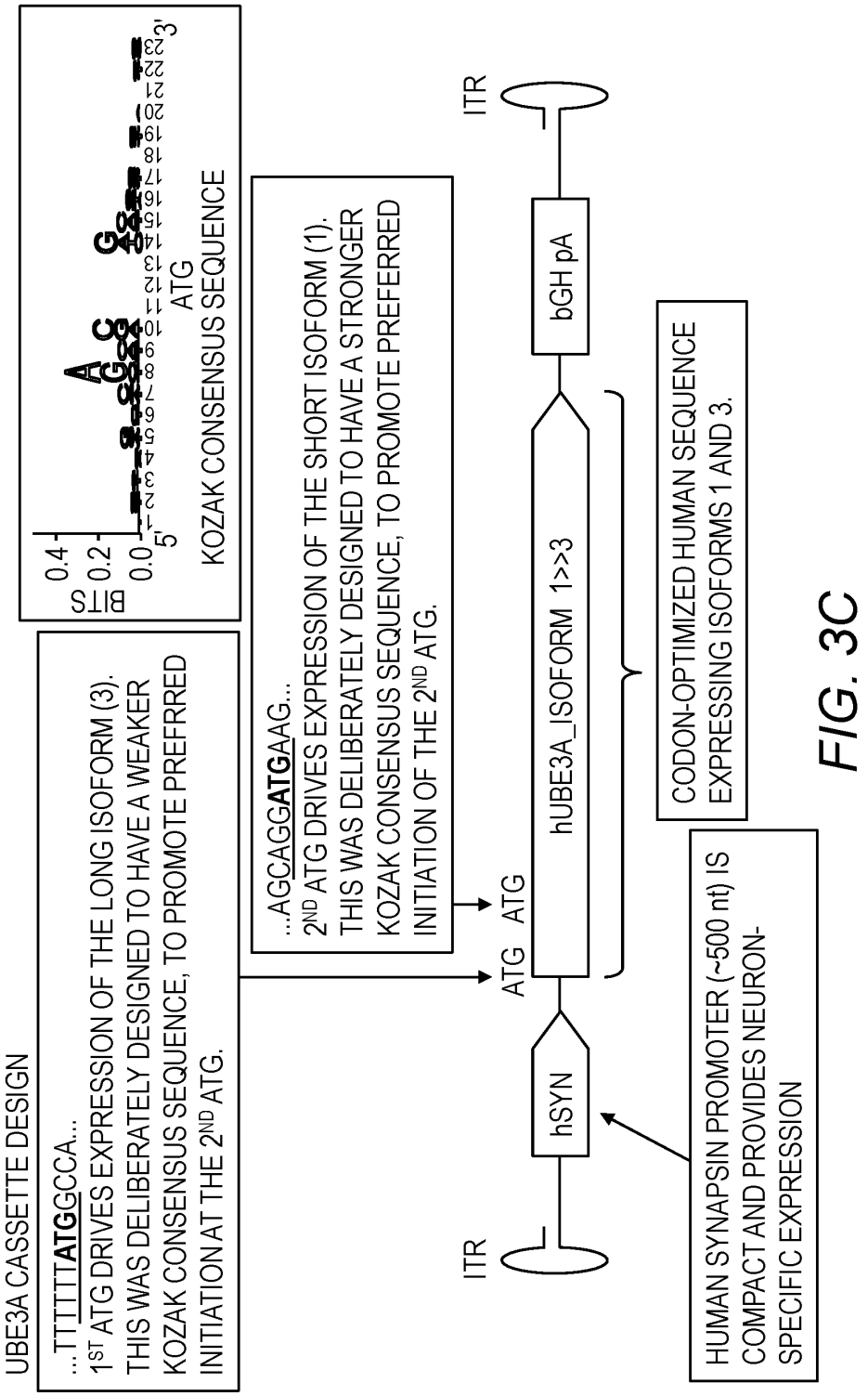

UBE3A CASSETTE DESIGN
...TTTTTTATGGCCA...
1ST ATG DRIVES EXPRESSION OF THE LONG ISOFORM (3).
THIS WAS DELIBERATELY DESIGNED TO HAVE A WEAKER KOZAK CONSENSUS SEQUENCE, TO PROMOTE PREFRRED INITIATION AT THE 2ND ATG.
...AGCAGGATGAAG...
2ND ATG DRIVES EXPRESSION OF THE SHORT ISOFORM (1).
THIS WAS DELIBERATELY DESIGNED TO HAVE A STRONGER KOZAK CONSENSUS SEQUENCE, TO PROMOTE PREFERRED INITIATION OF THE 2ND ATG.
BITS
0.4
0.2
0.0
5'
3'
ATG
KOZAK CONSENSUS SEQUENCE
ITR
hSYN
ATG ATG
hUBE3A_ISOFORM 1>>3
bGH pA
ITR
CODON-OPTIMIZED HUMAN SEQUENCE EXPRESSING ISOFORMS 1 AND 3.
HUMAN SYNAPSIN PROMOTER (~500 nt) IS COMPACT AND PROVIDES NEURON-SPECIFIC EXPRESSION

FIG. 3C

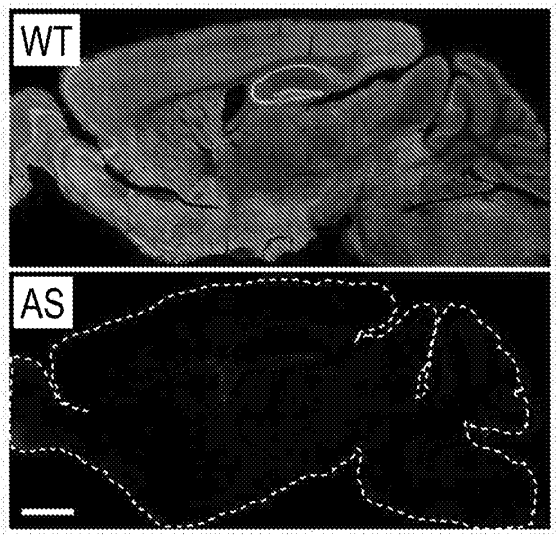


FIG. 4

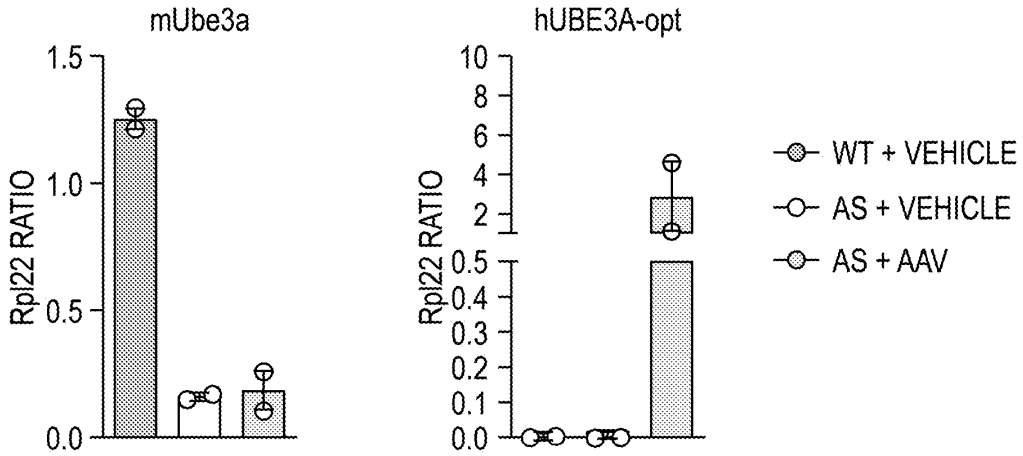


FIG. 5

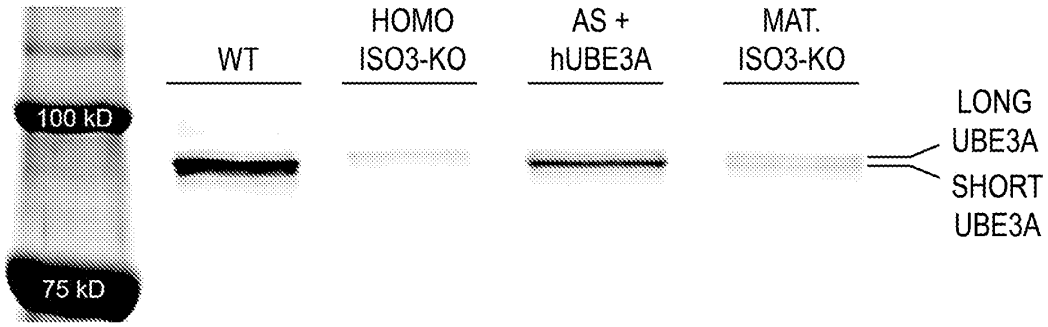


FIG. 6

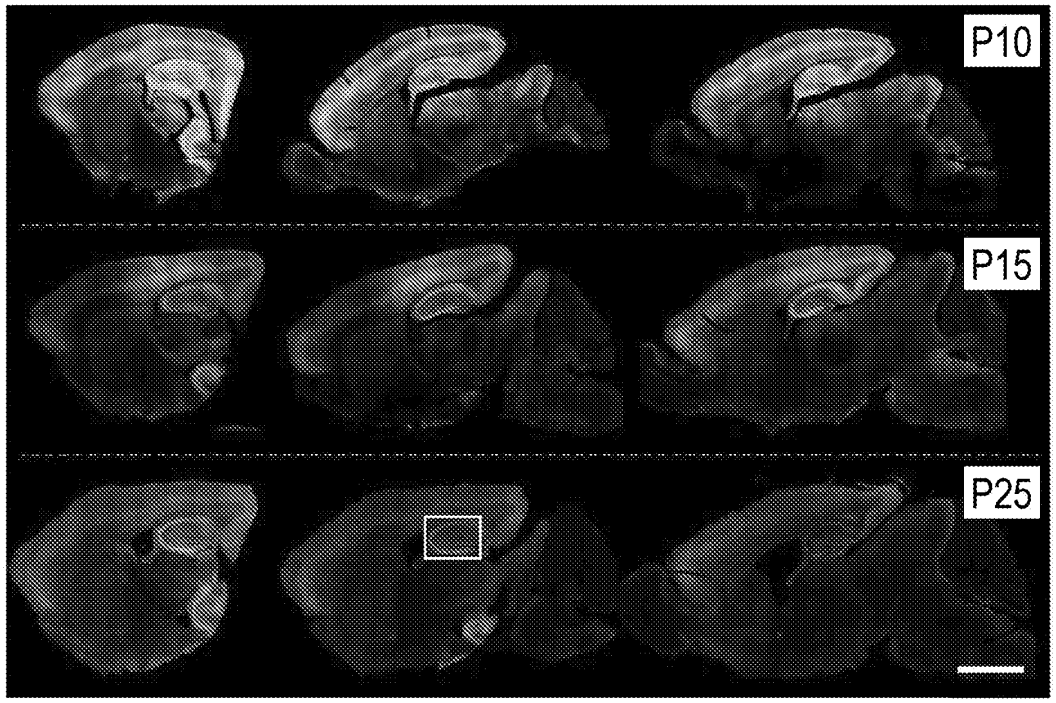


FIG. 7A

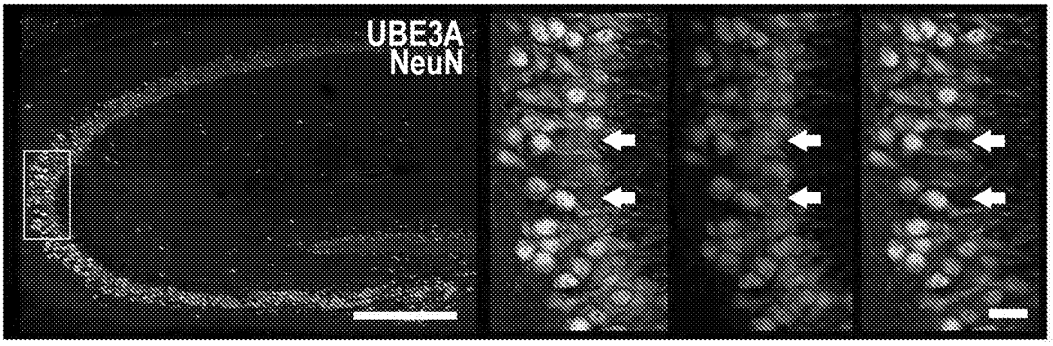


FIG. 7B

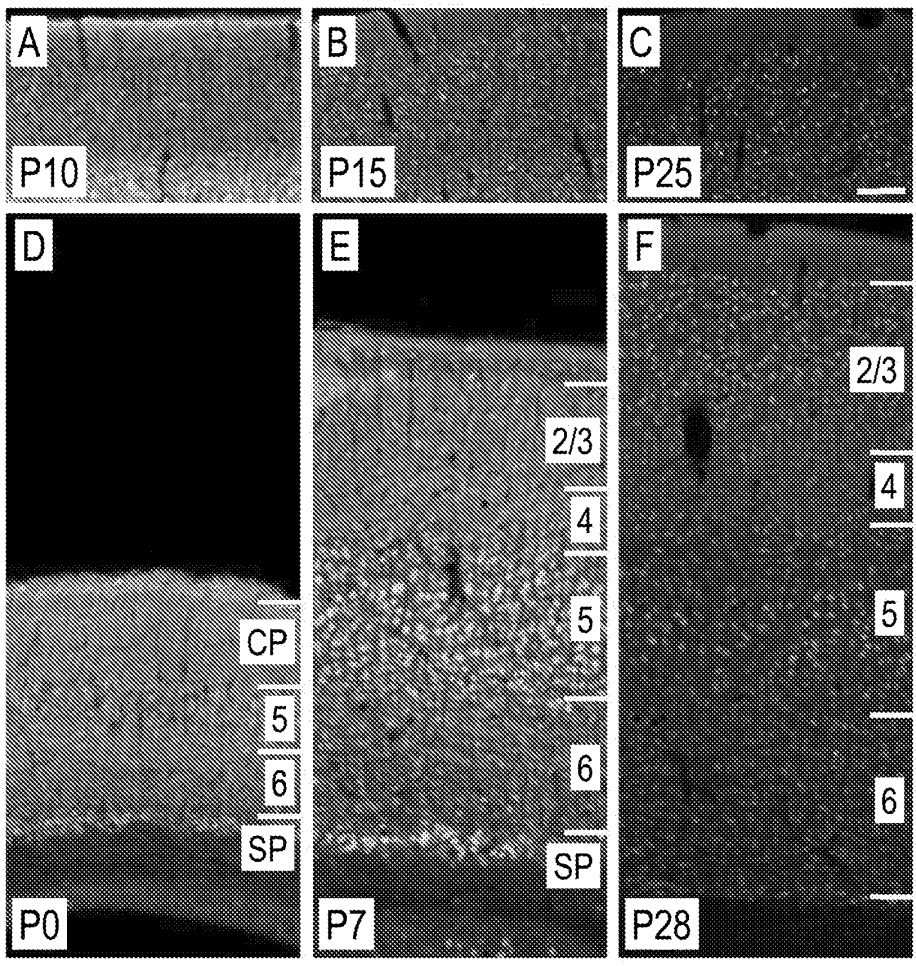

A
P10
B
P15
C
P25
D
CP
5
6
SP
P0
E
2/3
4
5
6
SP
P7
F
2/3
4
5
6
P28

FIG. 8

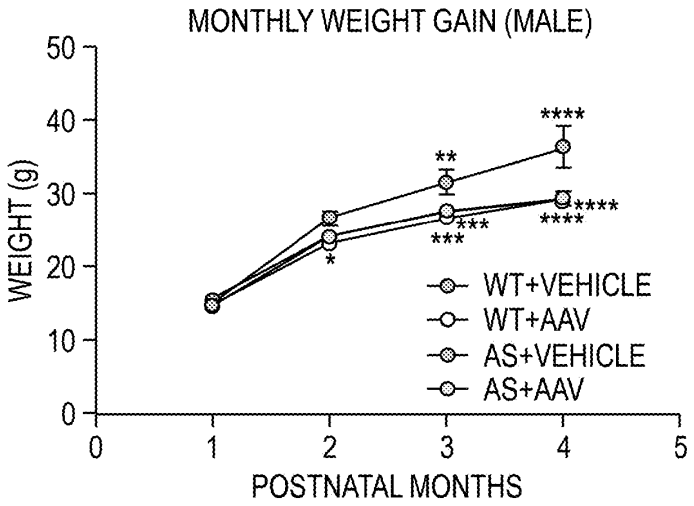

MONTHLY WEIGHT GAIN (MALE)
WEIGHT (g)
50
40
30
20
10
0
0
1
2
3
4
5
POSTNATAL MONTHS
WT+VEHICLE
WT+AAV
AS+VEHICLE
AS+AAV
*
**
***
****

FIG. 9

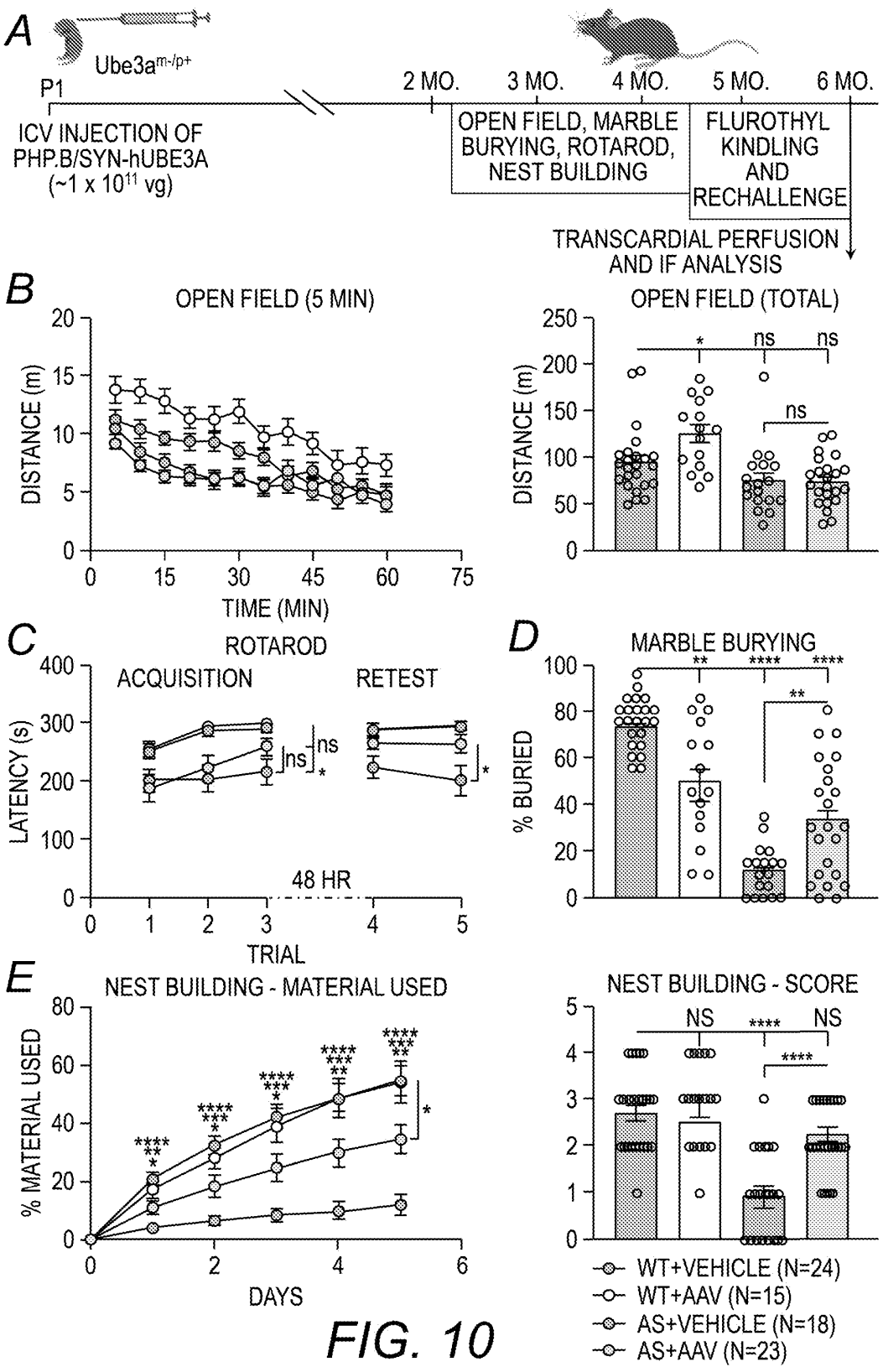

A
Ube3a m-/p+
P1
2 MO.
3 MO.
4 MO.
5 MO.
6 MO.
ICV INJECTION OF PHP.B/SYN-hUBE3A (~1 x 10^11 vg)
OPEN FIELD, MARBLE BURYING, ROTAROD, NEST BUILDING
FLUROTHYL KINDLING AND RECHALLENGE
TRANSCARDIAL PERFUSION AND IF ANALYSIS
B
OPEN FIELD (5 MIN)
DISTANCE (m)
TIME (MIN)
OPEN FIELD (TOTAL)
DISTANCE (m)
ns
C
ROTAROD
ACQUISITION
RETEST
LATENCY (s)
48 HR
TRIAL
D
MARBLE BURYING
% BURIED
E
NEST BUILDING - MATERIAL USED
% MATERIAL USED
DAYS
NEST BUILDING - SCORE
NS
WT+VEHICLE (N=24)
WT+AAV (N=15)
AS+VEHICLE (N=18)
AS+AAV (N=23)

FIG. 10

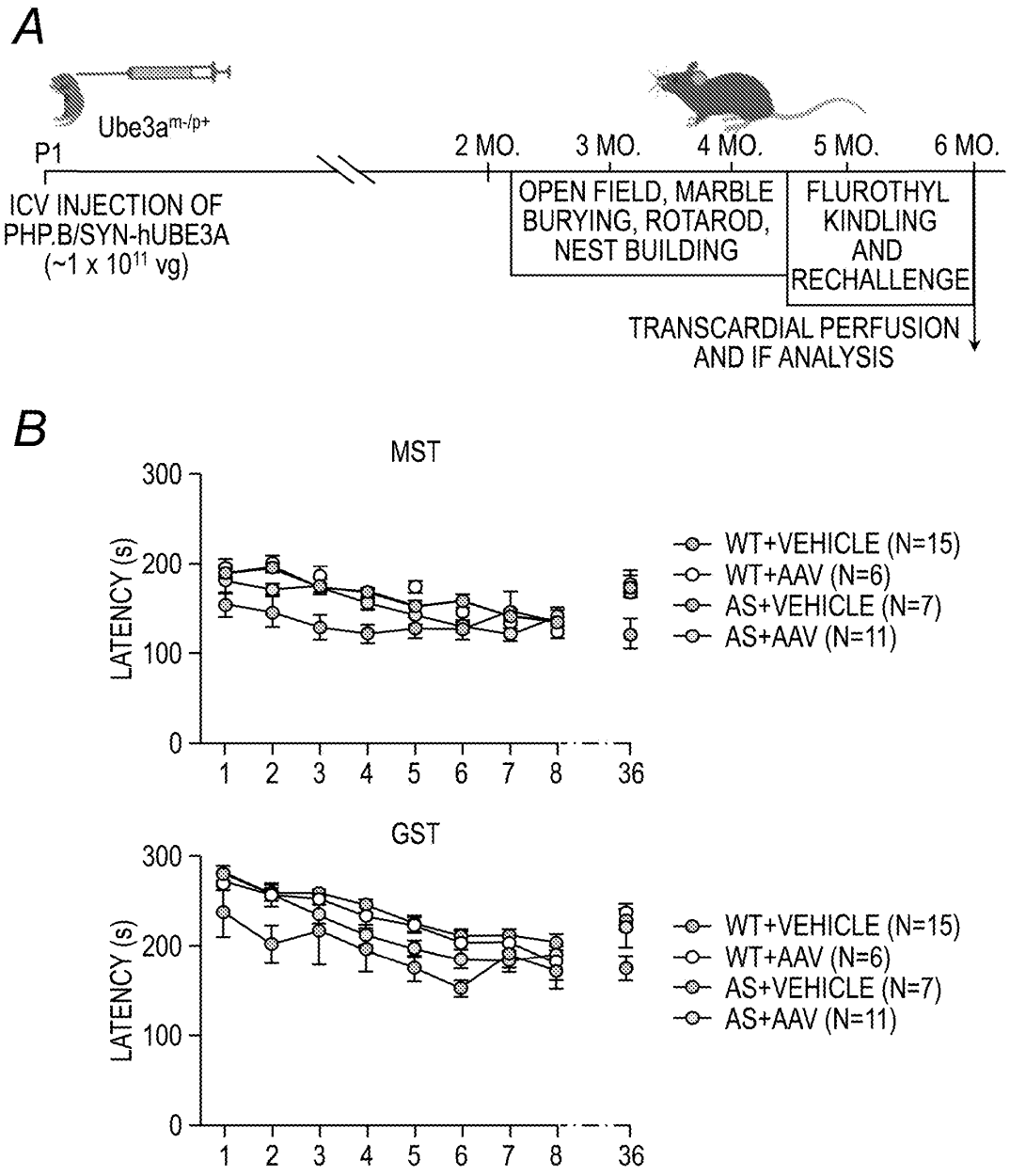

A
Ube3a$^{m-/p+}$
P1
2 MO.
3 MO.
4 MO.
5 MO.
6 MO.
ICV INJECTION OF PHP.B/SYN-hUBE3A (~1 x 10^{11} vg)
OPEN FIELD, MARBLE BURYING, ROTAROD, NEST BUILDING
FLUROTHYL KINDLING AND RECHALLENGE
TRANSCARDIAL PERFUSION AND IF ANALYSIS
B
MST
LATENCY (s)
300
200
100
0
1 2 3 4 5 6 7 8 36
WT+VEHICLE (N=15)
WT+AAV (N=6)
AS+VEHICLE (N=7)
AS+AAV (N=11)
GST
LATENCY (s)
300
200
100
0
1 2 3 4 5 6 7 8 36
WT+VEHICLE (N=15)
WT+AAV (N=6)
AS+VEHICLE (N=7)
AS+AAV (N=11)

FIG. 11

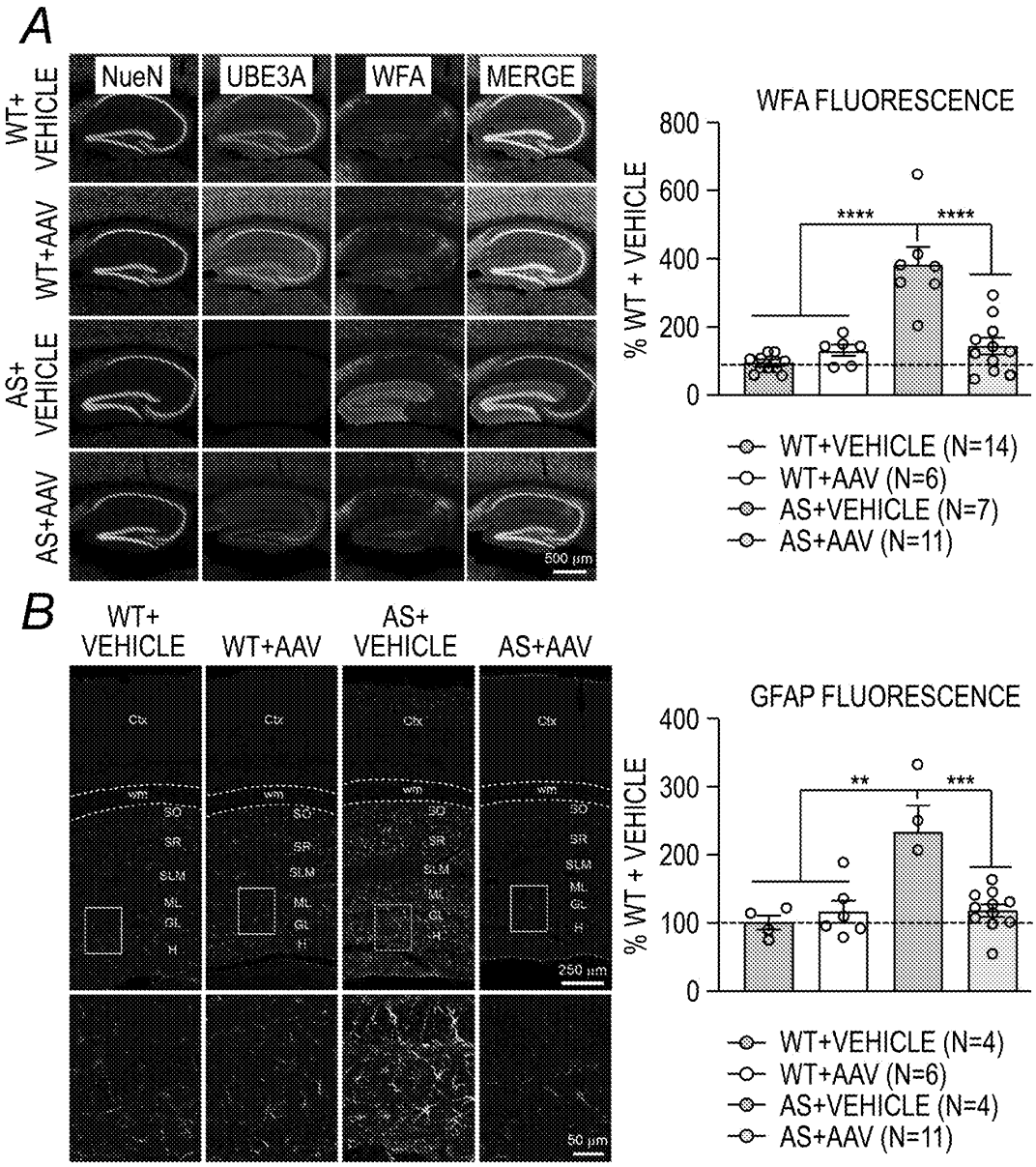

A
NueN
UBE3A
WFA
MERGE
WT+ VEHICLE
WT+AAV
AS+ VEHICLE
AS+AAV
500 µm
WFA FLUORESCENCE
% WT + VEHICLE
800
600
400
200
0
****
****
WT+VEHICLE (N=14)
WT+AAV (N=6)
AS+VEHICLE (N=7)
AS+AAV (N=11)
B
WT+ VEHICLE
WT+AAV
AS+ VEHICLE
AS+AAV
Ctx
wm
SO
SR
SLM
ML
GL
H
250 µm
50 µm
GFAP FLUORESCENCE
% WT + VEHICLE
400
300
200
100
0
**
***
WT+VEHICLE (N=4)
WT+AAV (N=6)
AS+VEHICLE (N=4)
AS+AAV (N=11)

FIG. 12

```
 721       CACC GAATGGCCAC AGCTTGTAAA AGATCAGGAG AACCTCAGTC TGACGACATT
 781 GAAGCTAGCC GAATGAAGCG AGCAGCTGCA AAGCATCTAA TAGAACGCTA CTACCACCAG
 841 TTAACTGAGG GCTGTGGAAA TGAAGCCTGC ACGAATGAGT TTTGTGCTTC CTGTCCAACT
 901 TTTCTTCGTA TGGATAATAA TGCAGCAGCT ATTAAAGCCC TCGAGCTTTA TAAGATTAAT
 961 GCAAAACTCT GTGATCCTCA TCCCTCCAAG AAAGGAGCAA GCTCAGCTTA CCTTGAGAAC
1021 TCGAAAGGTG CCCCCAACAA CTCCTGCTCT GAGATAAAAA TGAACAAGAA AGGCGCTAGA
1081 ATTGATTTTA AAGATGTGAC TTACTTAACA GAAGAGAAGG TATATGAAAT TCTTGAATTA
1141 TGTAGAGAAA GAGAGGATTA TTCCCCTTTA ATCCGTGTTA TTGGAAGAGT TTTTTCTAGT
1201 GCTGAGGCAT TGGTACAGAG CTTCCGGAAA GTTAAACAAC ACACCAAGGA AGAACTGAAA
1261 TCTCTTCAAG CAAAAGATGA AGACAAAGAT GAAGATGAAA AGGAAAAAGC TGCATGTTCT
1321 GCTGCTGCTA TGGAAGAAGA CTCAGAAGCA TCTTCCTCAA GGATAGGTGA TAGCTCACAG
1381 GGAGACAACA ATTTGCAAAA ATTAGGCCCT GATGATGTGT CTGTGGATAT TGATGCCATT
1441 AGAAGGGTCT ACACCAGATT GCTCTCTAAT GAAAAAATTG AAACTGCCTT TCTCAATGCA
1501 CTTGTATATT TGTCACCTAA CGTGGAATGT GACTTGACGT ATCACAATGT ATACTCTCGA
1561 GATCCTAATT ATCTGAATTT GTTCATTATC GTAATGGAGA ATAGAAATCT CCACAGTCCT
1621 GAATATCTGG AAATGGCTTT GCCATTATTT TGCAAAGCGA TGAGCAAGCT ACCCCTTGCA
1681 GCCCAAGGAA AACTGATCAG ACTGTGGTCT AAATACAATG CAGACCAGAT TCGGAGAATG
1741 ATGGAGACAT TTCAGCAACT TATTACTTAT AAAGTCATAA GCAATGAATT TAACAGTCGA
1801 AATCTAGTGA ATGATGATGA TGCCATTGTT GCTGCTTCGA AGTGCTTGAA AATGGTTTAC
1861 TATGCAAATG TAGTGGGAGG GGAAGTGGAC ACAAATCACA ATGAAGAAGA TGATGAAGAG
1921 CCCATCCCTG AGTCCAGCGA GCTGACACTT CAGGAACTTT TGGGAGAAGA AAGAAGAAAC
1981 AAGAAAGGTC CTCGAGTGGA CCCCCTGGAA ACTGAACTTG GTGTTAAAAC CCTGGATTGT
2041 CGAAAACCAC TTATCCCTTT TGAAGAGTTT ATTAATGAAC CACTGAATGA GGTTCTAGAA
2101 ATGGATAAAG ATTATACTTT TTTCAAAGTA GAAACAGAGA ACAAATTCTC TTTTATGACA
2161 TGTCCCTTTA TATTGAATGC TGTCACAAAG AATTTGGGAT TATATTATGA CAATAGAATT
2221 CGCATGTACA GTGAACGAAG AATCACTGTT CTCTACAGCT TAGTTCAAGG ACAGCAGTTG
2281 AATCCATATT TGAGACTCAA AGTTAGACGT GACCATATCA TAGATGATGC ACTTGTCCGG
2341 CTAGAGATGA TCGCTATGGA AAATCCTGCA GACTTGAAGA AGCAGTTGTA TGTGGAATTT
2401 GAAGGAGAAC AAGGAGTTGA TGAGGGAGGT GTTTCCAAAG AATTTTTTCA GCTGGTTGTG
2461 GAGGAAATCT TCAATCCAGA TATTGGTATG TTCACATACG ATGAATCTAC AAAATTGTTT
2521 TGGTTTAATC CATCTTCTTT TGAAACTGAG GGTCAGTTTA CTCTGATTGG CATAGTACTG
2581 GGTCTGGCTA TTTACAATAA CTGTATACTG GATGTACATT TTCCCATGGT TGTCTACAGG
2641 AAGCTAATGG GGAAAAAAGG AACTTTTCGT GACTTGGGAG ACTCTCACCC AGTTCTATAT
2701 CAGAGTTTAA AAGATTTATT GGAGTATGAA GGGAATGTGG AAGATGACAT GATGATCACT
2761 TTCCAGATAT CACAGACAGA TCTTTTTGGT AACCCAATGA TGTATGATCT AAAGGAAAAT
2821 GGTGATAAAA TTCCAATTAC AAATGAAAAC AGGAAGGAAT TTGTCAATCT TTATTCTGAC
2881 TACATTCTCA ATAAATCAGT AGAAAAACAG TTCAAGGCTT TTCGGAGAGG TTTTCATATG
2941 GTGACCAATG AATCTCCCTT AAAGTACTTA TTCAGACCAG AAGAAATTGA ATTGCTTATA
3001 TGTGGAAGCC GGAATCTAGA TTTCCAAGCA CTAGAAGAAA CTACAGAATA TGACGGTGGC
3061 TATACCAGGG ACTCTGTTCT GATTAGGGAG TTCTGGGAAA TCGTTCATTC ATTTACAGAT
3121 GAACAGAAAA GACTCTTCTT GCAGTTTACA ACGGGCACAG ACAGAGCACC TGTGGGAGGA
```

FIG. 13

```
 721       TTTT TTATGGCCAC AGCTTGTAAA AGATCAGGAG AACCTCAGTC TGACGACATT
 781 GAAGCTAGCA GGATGAAGCG AGCAGCTGCA AAGCATCTAA TAGAACGCTA CTACCACCAG
 841 TTAACTGAGG GCTGTGGAAA TGAAGCCTGC ACGAATGAGT TTTGTGCTTC CTGTCCAACT
 901 TTTCTTCGTA TGGATAATAA TGCAGCAGCT ATTAAAGCCC TCGAGCTTTA TAAGATTAAT
 961 GCAAAACTCT GTGATCCTCA TCCCTCCAAG AAAGGAGCAA GCTCAGCTTA CCTTGAGAAC
1021 TCGAAAGGTG CCCCCAACAA CTCCTGCTCT GAGATAAAAA TGAACAAGAA AGGCGCTAGA
1081 ATTGATTTTA AAGATGTGAC TTACTTAACA GAAGAGAAGG TATATGAAAT TCTTGAATTA
1141 TGTAGAGAAA GAGAGGATTA TTCCCCTTTA ATCCGTGTTA TTGGAAGAGT TTTTTCTAGT
1201 GCTGAGGCAT TGGTACAGAG CTTCCGGAAA GTTAAACAAC ACACCAAGGA AGAACTGAAA
1261 TCTCTTCAAG CAAAAGATGA AGACAAAGAT GAAGATGAAA AGGAAAAAGC TGCATGTTCT
1321 GCTGCTGCTA TGGAAGAAGA CTCAGAAGCA TCTTCCTCAA GGATAGGTGA TAGCTCACAG
1381 GGAGACAACA ATTTGCAAAA ATTAGGCCCT GATGATGTGT CTGTGGATAT TGATGCCATT
1441 AGAAGGGTCT ACACCAGATT GCTCTCTAAT GAAAAAATTG AAACTGCCTT TCTCAATGCA
1501 CTTGTATATT TGTCACCTAA CGTGGAATGT GACTTGACGT ATCACAATGT ATACTCTCGA
1561 GATCCTAATT ATCTGAATTT GTTCATTATC GTAATGGAGA ATAGAAATCT CCACAGTCCT
1621 GAATATCTGG AAATGGCTTT GCCATTATTT TGCAAAGCGA TGAGCAAGCT ACCCCTTGCA
1681 GCCCAAGGAA AACTGATCAG ACTGTGGTCT AAATACAATG CAGACCAGAT TCGGAGAATG
1741 ATGGAGACAT TTCAGCAACT TATTACTTAT AAAGTCATAA GCAATGAATT TAACAGTCGA
1801 AATCTAGTGA ATGATGATGA TGCCATTGTT GCTGCTTCGA AGTGCTTGAA AATGGTTTAC
1861 TATGCAAATG TAGTGGGAGG GGAAGTGGAC ACAAATCACA ATGAAGAAGA TGATGAAGAG
1921 CCCATCCCTG AGTCCAGCGA GCTGACACTT CAGGAACTTT TGGGAGAAGA AAGAAGAAAC
1981 AAGAAAGGTC CTCGAGTGGA CCCCCTGGAA ACTGAACTTG GTGTTAAAAC CCTGGATTGT
2041 CGAAAACCAC TTATCCCTTT TGAAGAGTTT ATTAATGAAC CACTGAATGA GGTTCTAGAA
2101 ATGGATAAAG ATTATACTTT TTTCAAAGTA GAAACAGAGA ACAAATTCTC TTTTATGACA
2161 TGTCCCTTTA TATTGAATGC TGTCACAAAG AATTTGGGAT TATATTATGA CAATAGAATT
2221 CGCATGTACA GTGAACGAAG AATCACTGTT CTCTACAGCT TAGTTCAAGG ACAGCAGTTG
2281 AATCCATATT TGAGACTCAA AGTTAGACGT GACCATATCA TAGATGATGC ACTTGTCCGG
2341 CTAGAGATGA TCGCTATGGA AAATCCTGCA GACTTGAAGA AGCAGTTGTA TGTGGAATTT
2401 GAAGGAGAAC AAGGAGTTGA TGAGGGAGGT GTTTCCAAAG AATTTTTTCA GCTGGTTGTG
2461 GAGGAAATCT TCAATCCAGA TATTGGTATG TTCACATACG ATGAATCTAC AAAATTGTTT
2521 TGGTTTAATC CATCTTCTTT TGAAACTGAG GGTCAGTTTA CTCTGATTGG CATAGTACTG
2581 GGTCTGGCTA TTTACAATAA CTGTATACTG GATGTACATT TTCCCATGGT TGTCTACAGG
2641 AAGCTAATGG GGAAAAAAGG AACTTTTCGT GACTTGGGAG ACTCTCACCC AGTTCTATAT
2701 CAGAGTTTAA AAGATTTATT GGAGTATGAA GGGAATGTGG AAGATGACAT GATGATCACT
2761 TTCCAGATAT CACAGACAGA TCTTTTTGGT AACCCAATGA TGTATGATCT AAAGGAAAAT
2821 GGTGATAAAA TTCCAATTAC AAATGAAAAC AGGAAGGAAT TTGTCAATCT TTATTCTGAC
2881 TACATTCTCA ATAAATCAGT AGAAAAACAG TTCAAGGCTT TTCGGAGAGG TTTTCATATG
2941 GTGACCAATG AATCTCCCTT AAAGTACTTA TTCAGACCAG AAGAAATTGA ATTGCTTATA
3001 TGTGGAAGCC GGAATCTAGA TTTCCAAGCA CTAGAAGAAA CTACAGAATA TGACGGTGGC
3061 TATACCAGGG ACTCTGTTCT GATTAGGGAG TTCTGGGAAA TCGTTCATTC ATTTACAGAT
3121 GAACAGAAAA GACTCTTCTT GCAGTTTACA ACGGGCACAG ACAGAGCACC TGTGGGAGGA
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FIG. 14