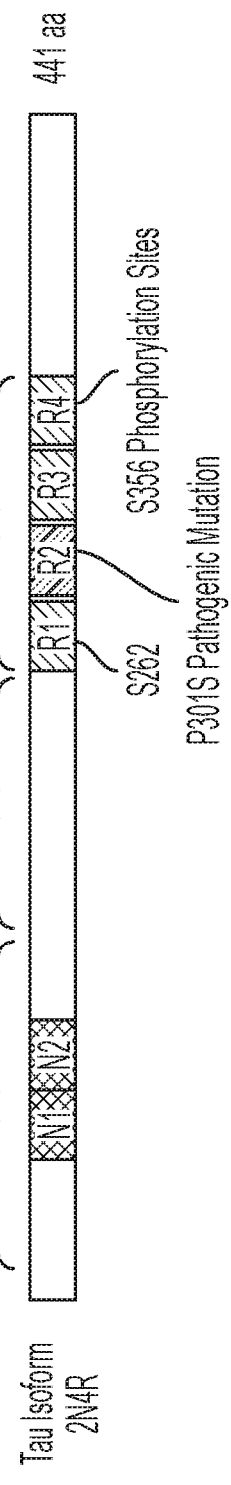
Projection Domain
Proline Rich Domain
Microtubule Binding Domain
441 aa
Tau Isoform 2N4R
N1
N2
R1
R2
R3
R4
S262
S356 Phosphorylation Sites
P301S Pathogenic Mutation

FIG. 1

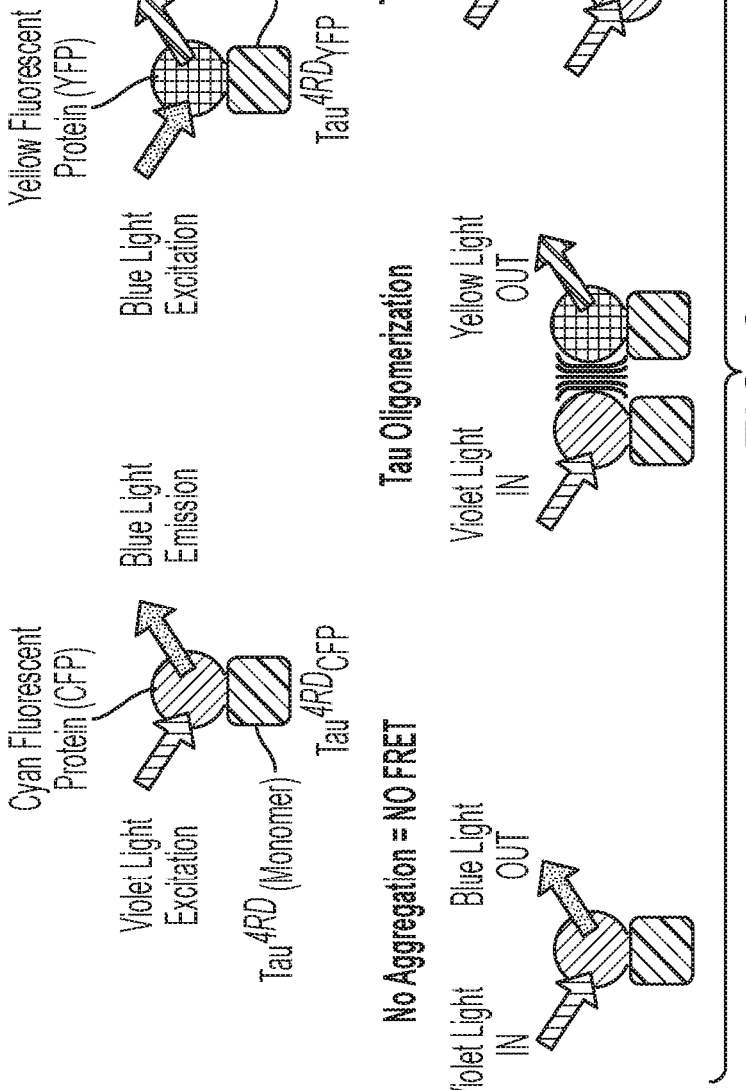
Cyan Fluorescent Protein (CFP)
Violet Light Excitation
Blue Light Emission
Tau4RD (Monomer)
Tau4RDCFP
Yellow Fluorescent Protein (YFP)
Blue Light Excitation
Yellow Light Emission
Tau4RD (Monomer)
Tau4RDYFP
No Aggregation = NO FRET
Violet Light IN
Blue Light OUT
Tau Oligomerization
Violet Light IN
Yellow Light OUT
Tau Aggregation = FRET

FIG. 2

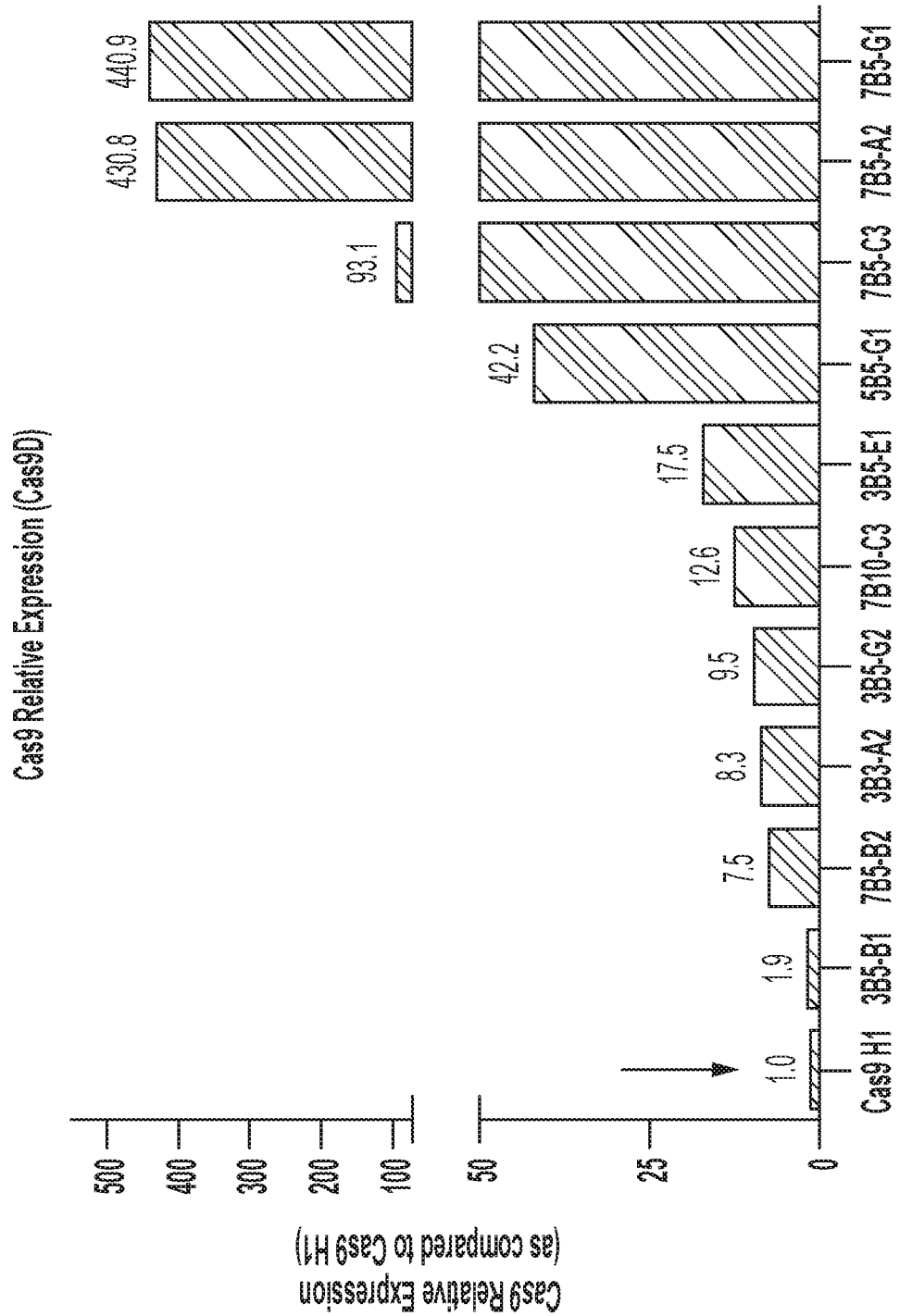
Cas9 Relative Expression (Cas9D)
Cas9 Relative Expression
(as compared to Cas9 H1)
500
400
300
200
100
50
25
0
1.0
1.9
7.5
8.3
9.5
12.6
17.5
42.2
93.1
430.8
440.9
Cas9 H1
3B5-B1
7B5-B2
3B3-A2
3B5-G2
7B10-C3
3B5-E1
5B5-G1
7B5-C3
7B5-A2
7B5-G1
TCY Cas9 Clones

FIG. 3A

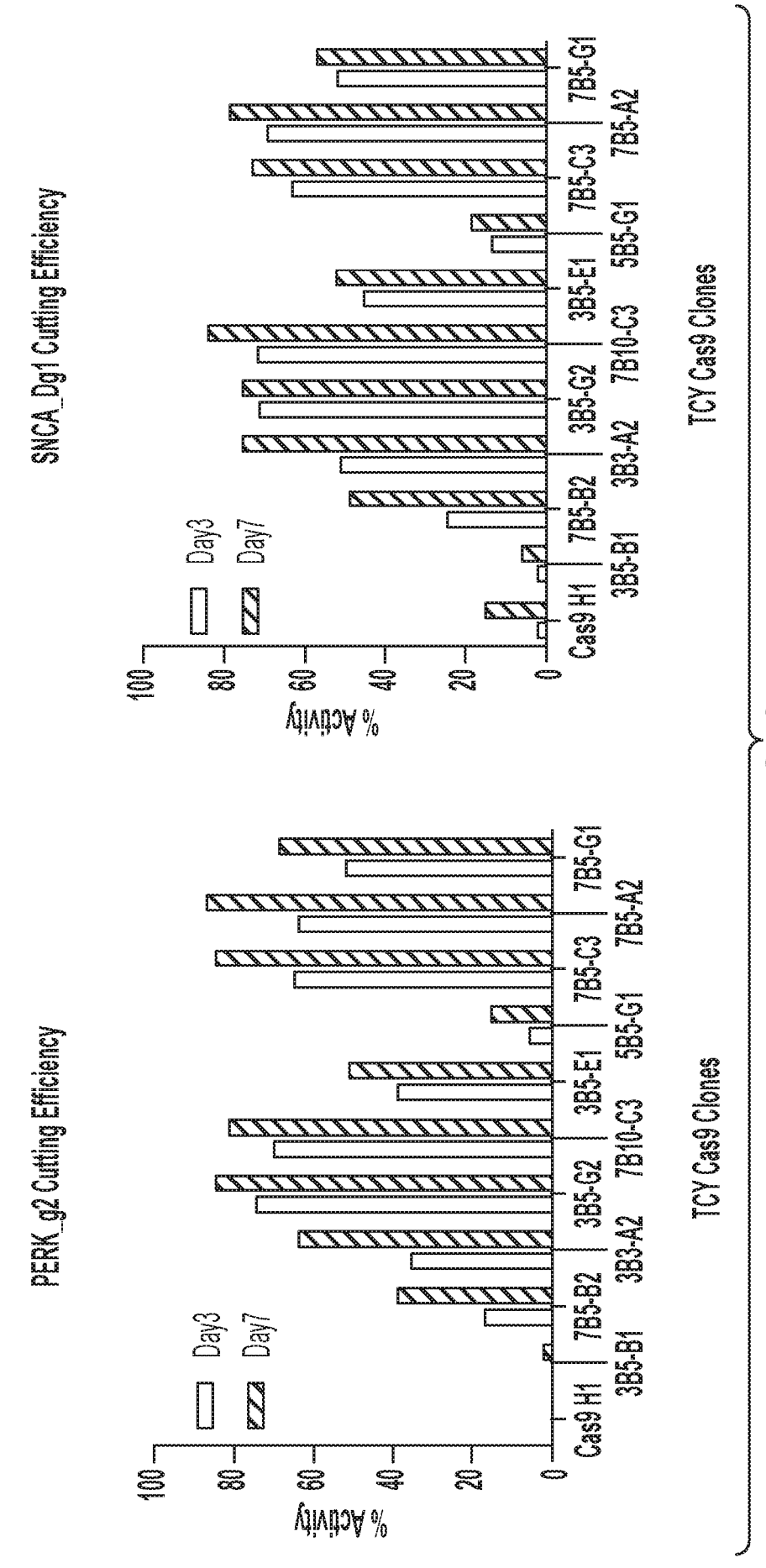
PERK_g2 Cutting Efficiency
% Activity
100
80
60
40
20
0
Day3
Day7
Cas9 H1
3B5-B1
7B5-B2
3B3-A2
3B5-G2
7B10-C3
3B5-E1
5B5-G1
7B5-C3
7B5-A2
7B5-G1
TCY Cas9 Clones
SNCA_Dg1 Cutting Efficiency
% Activity
100
80
60
40
20
0
Day3
Day7
Cas9 H1
3B5-B1
7B5-B2
3B3-A2
3B5-G2
7B10-C3
3B5-E1
5B5-G1
7B5-C3
7B5-A2
7B5-G1
TCY Cas9 Clones

FIG. 3B

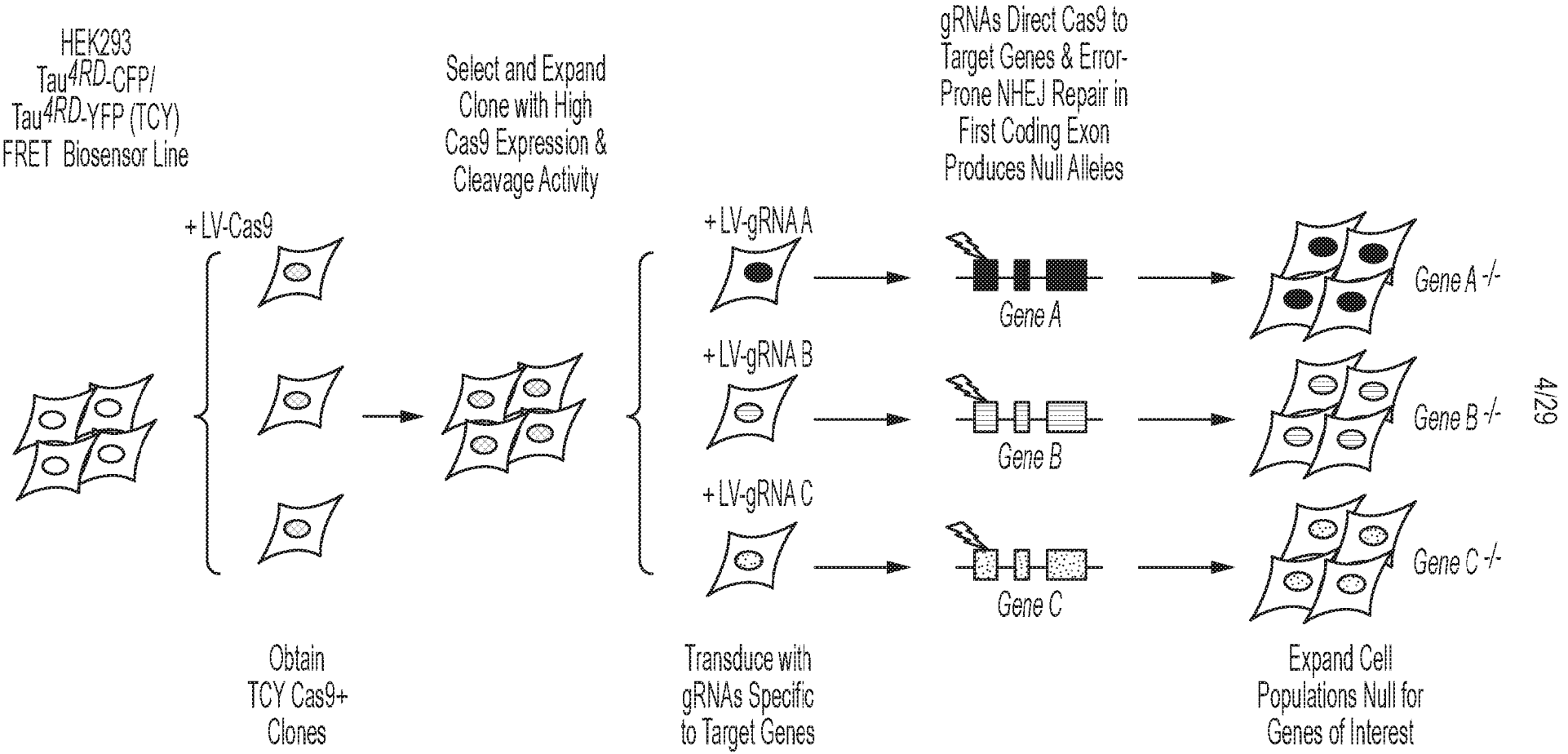
HEK293
Tau4RD-CFP/
Tau4RD-YFP (TCY)
FRET Biosensor Line
+ LV-Cas9
Obtain
TCY Cas9+
Clones
Select and Expand
Clone with High
Cas9 Expression &
Cleavage Activity
+ LV-gRNA A
+ LV-gRNA B
+ LV-gRNA C
Transduce with
gRNAs Specific
to Target Genes
gRNAs Direct Cas9 to
Target Genes & Error-
Prone NHEJ Repair in
First Coding Exon
Produces Null Alleles
Gene A
Gene B
Gene C
Gene A -/-
Gene B -/-
Gene C -/-
Expand Cell
Populations Null for
Genes of Interest

FIG. 4

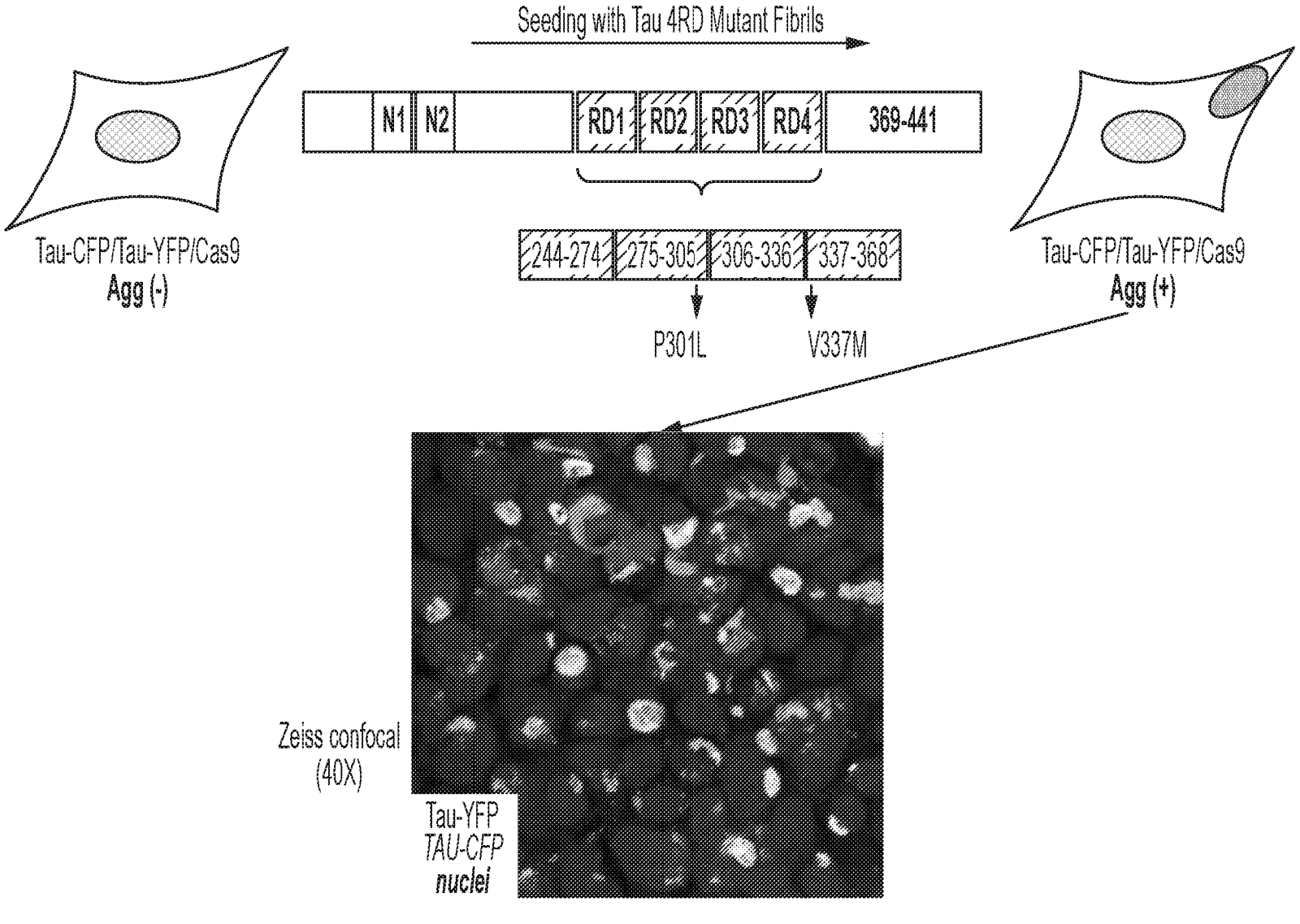
Seeding with Tau 4RD Mutant Fibrils
N1
N2
RD1
RD2
RD3
RD4
369-441
244-274
275-305
306-336
337-368
P301L
V337M
Tau-CFP/Tau-YFP/Cas9
Agg (-)
Tau-CFP/Tau-YFP/Cas9
Agg (+)
Zeiss confocal
(40X)
Tau-YFP
TAU-CFP
nuclei

FIG. 5

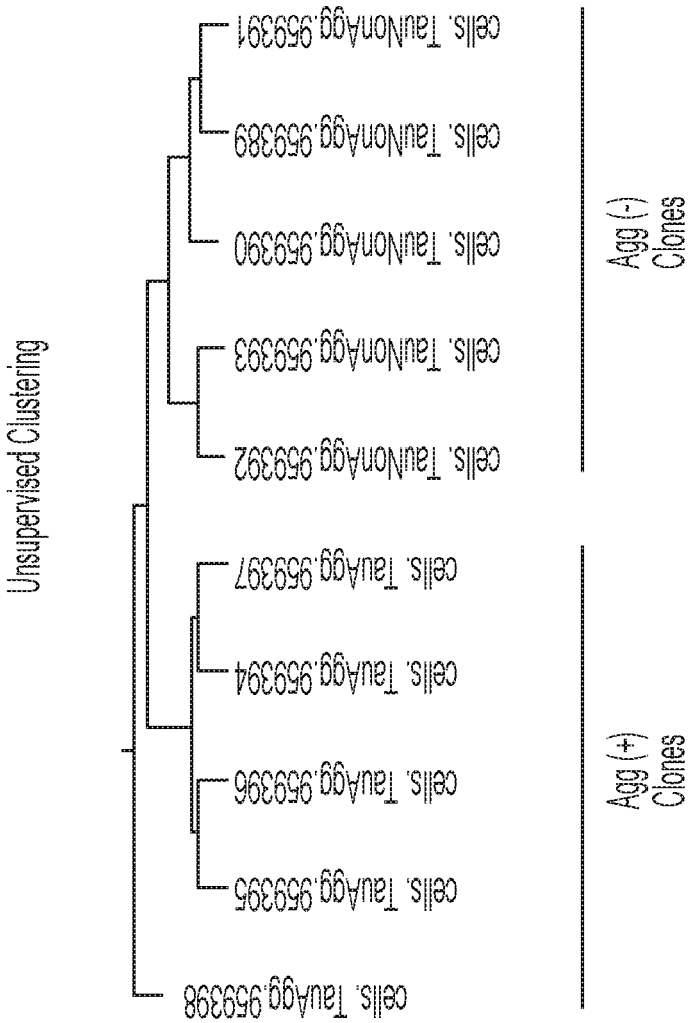
Unsupervised Clustering
cells.TauAgg.959398
cells.TauAgg.959395
cells.TauAgg.959396
cells.TauAgg.959394
cells.TauAgg.959397
cells.TauNonAgg.959392
cells.TauNonAgg.959393
cells.TauNonAgg.959390
cells.TauNonAgg.959389
cells.TauNonAgg.959391
Agg (+) Clones
Agg (-) Clones

FIG. 7

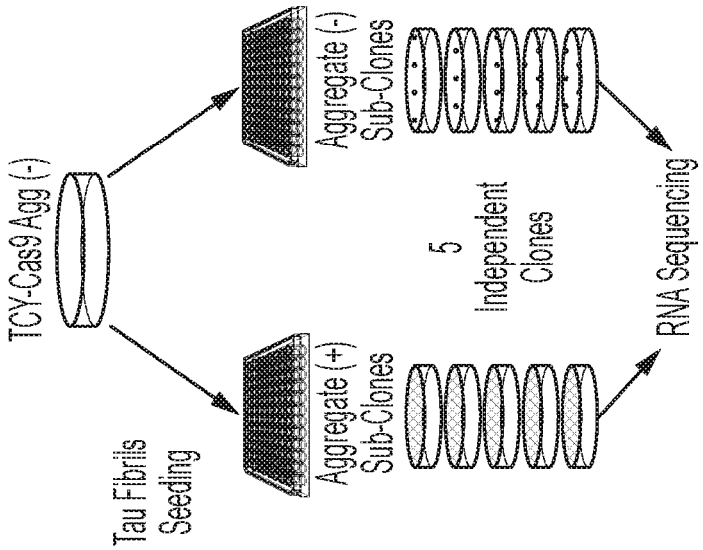
TCY-Cas9 Agg (-)
Tau Fibrils Seeding
Aggregate (+) Sub-Clones
Aggregate (-) Sub-Clones
5 Independent Clones
RNA Sequencing

FIG. 6

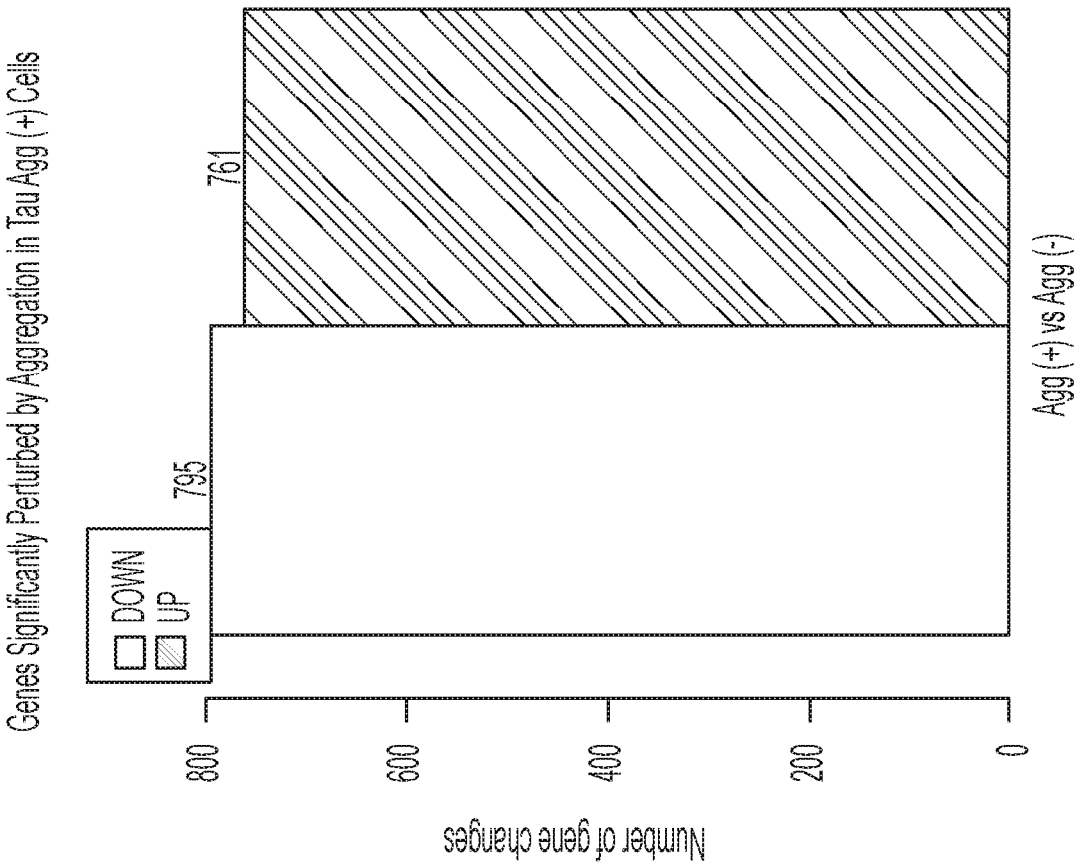
Genes Significantly Perturbed by Aggregation in Tau Agg (+) Cells
DOWN
UP
795
761
800
600
400
200
0
Number of gene changes
Agg (+) vs Agg (-)

FIG. 8

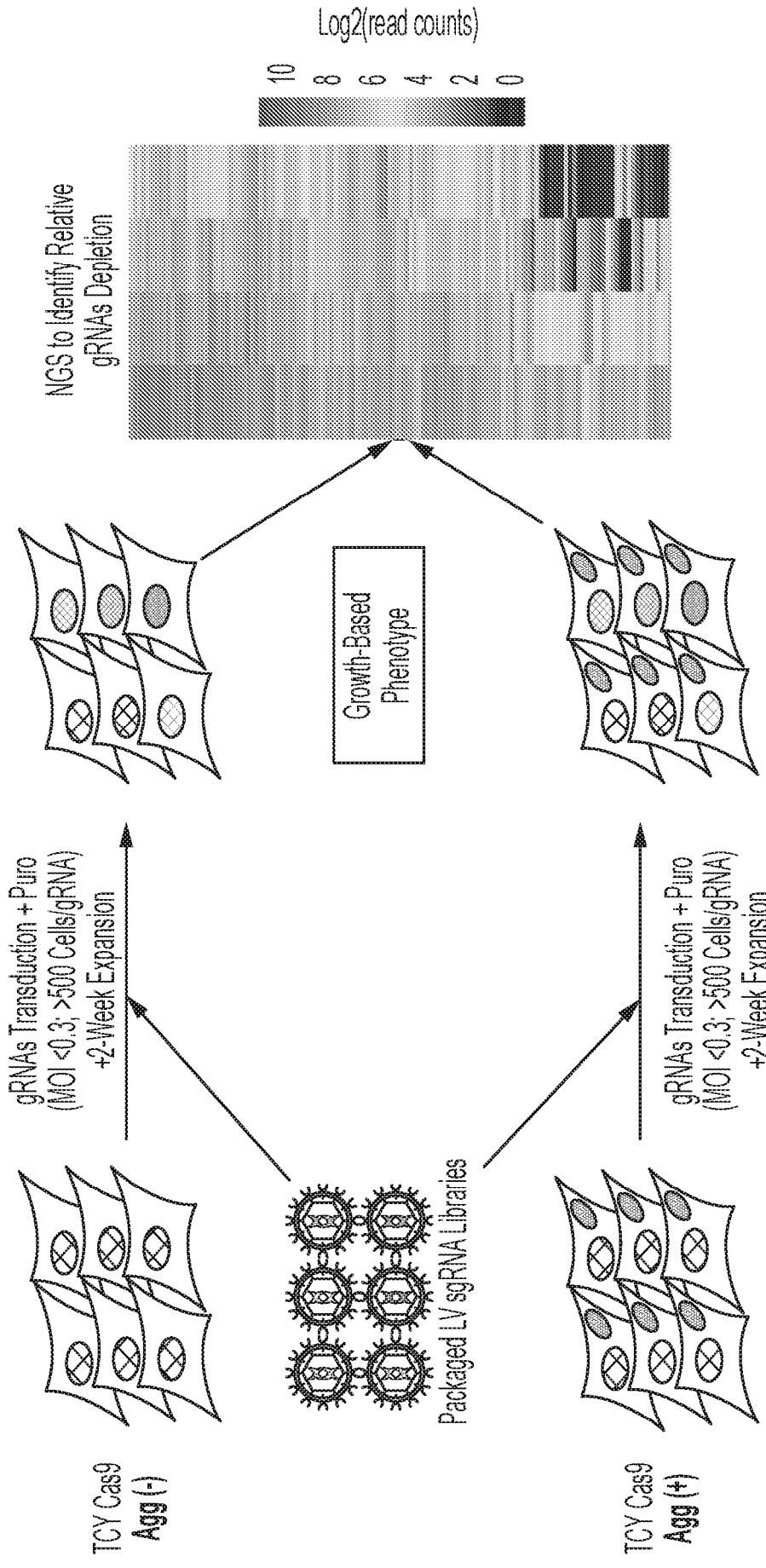
Log2(read counts)
10
8
6
4
2
0
NGS to Identify Relative gRNAs Depletion
Growth-Based Phenotype
gRNAs Transduction + Puro (MOI <0.3; >500 Cells/gRNA) +2-Week Expansion
gRNAs Transduction + Puro (MOI <0.3; >500 Cells/gRNA) +2-Week Expansion
TCY Cas9 Agg (-)
Packaged LV sgRNA Libraries
TCY Cas9 Agg (+)

FIG. 9

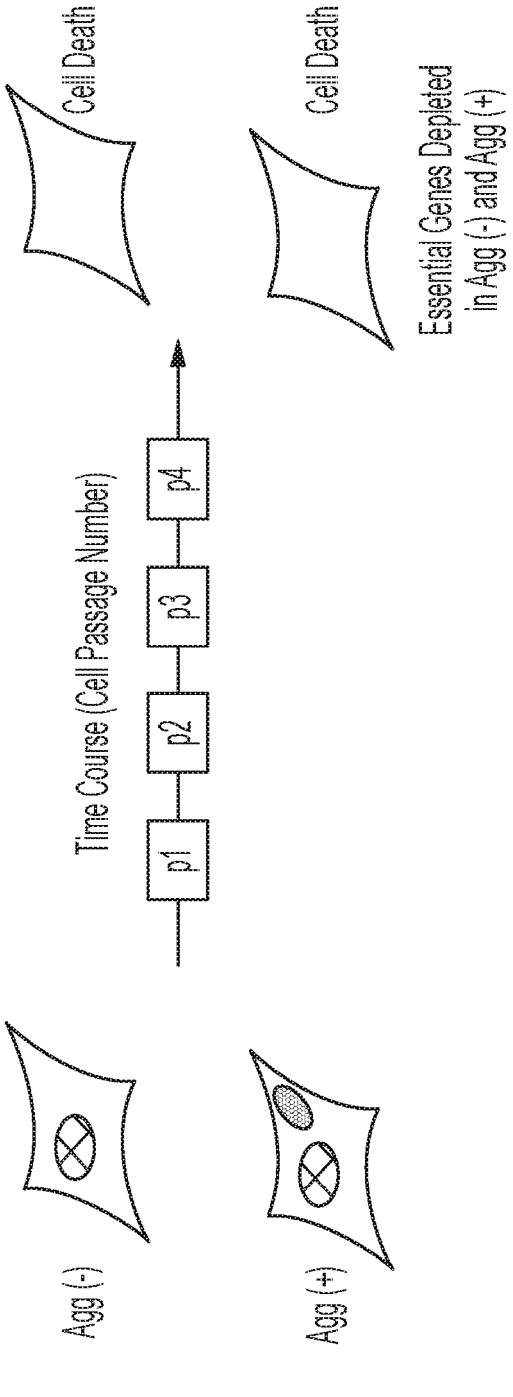

FIG. 10A

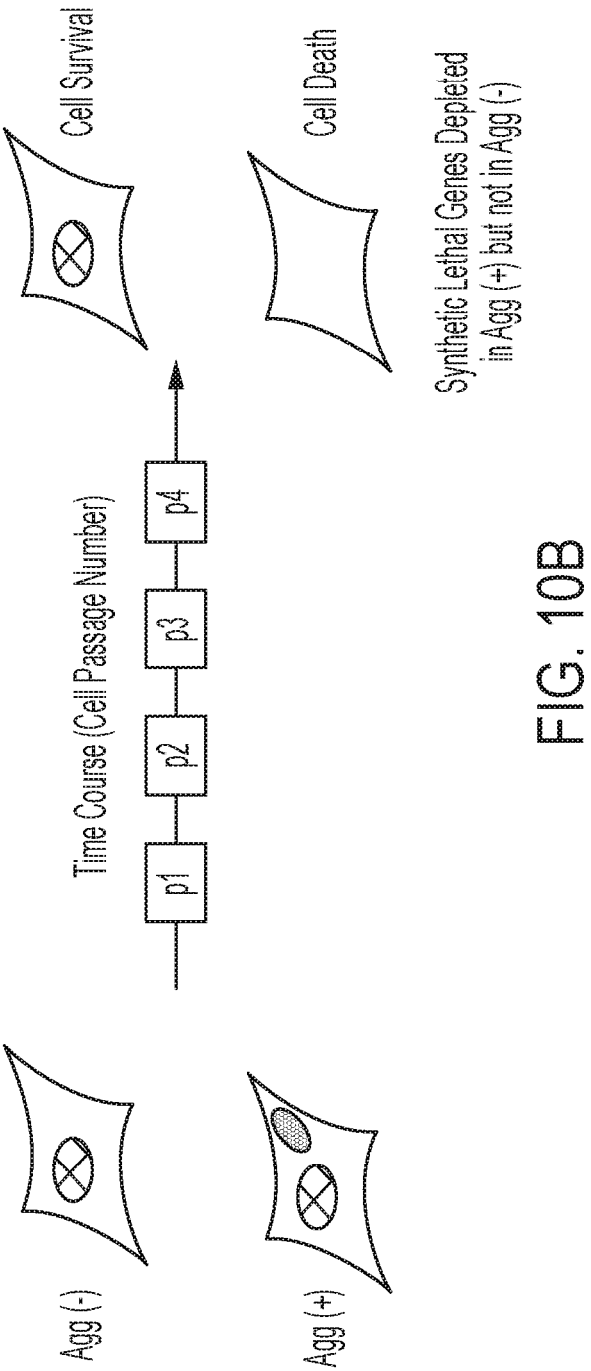

FIG. 10B

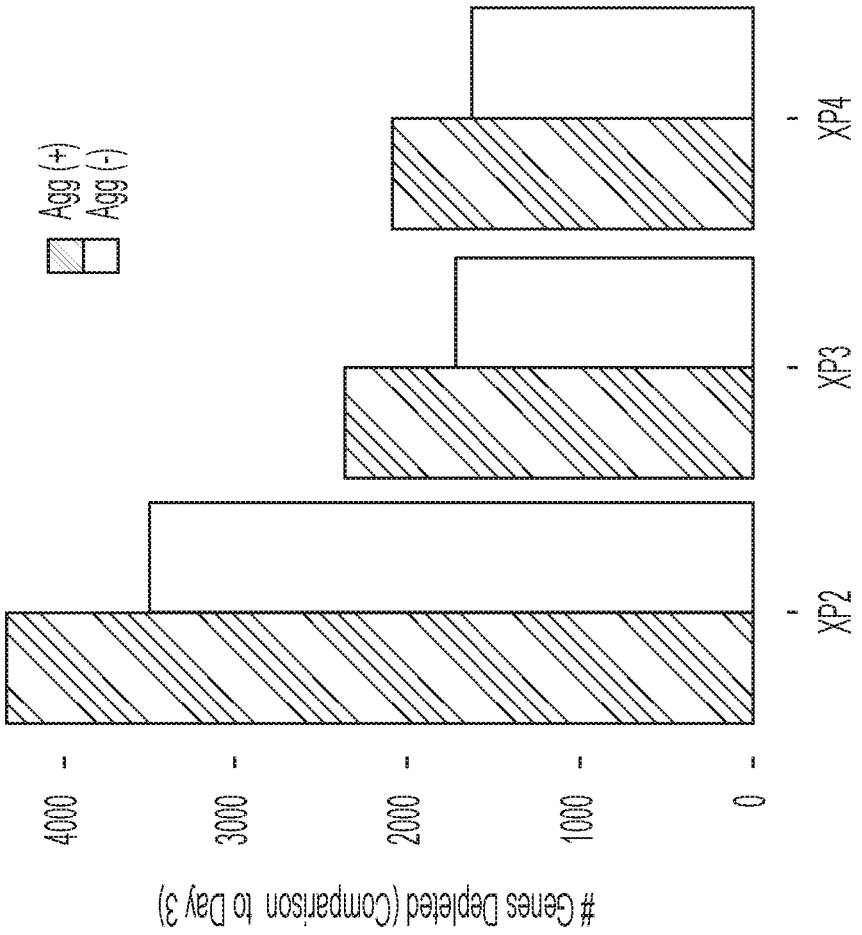
Agg (+)
Agg (-)
4000
3000
2000
1000
0
Genes Depleted (Comparison to Day 3)
XP2
XP3
XP4
XP Replicates

FIG. 12

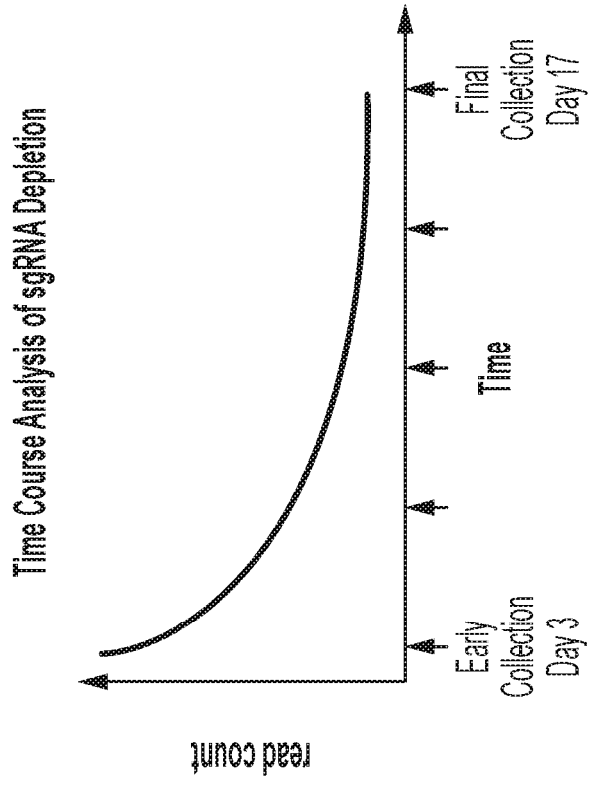
Time Course Analysis of sgRNA Depletion
read count
Early Collection Day 3
Time
Final Collection Day 17

FIG. 11

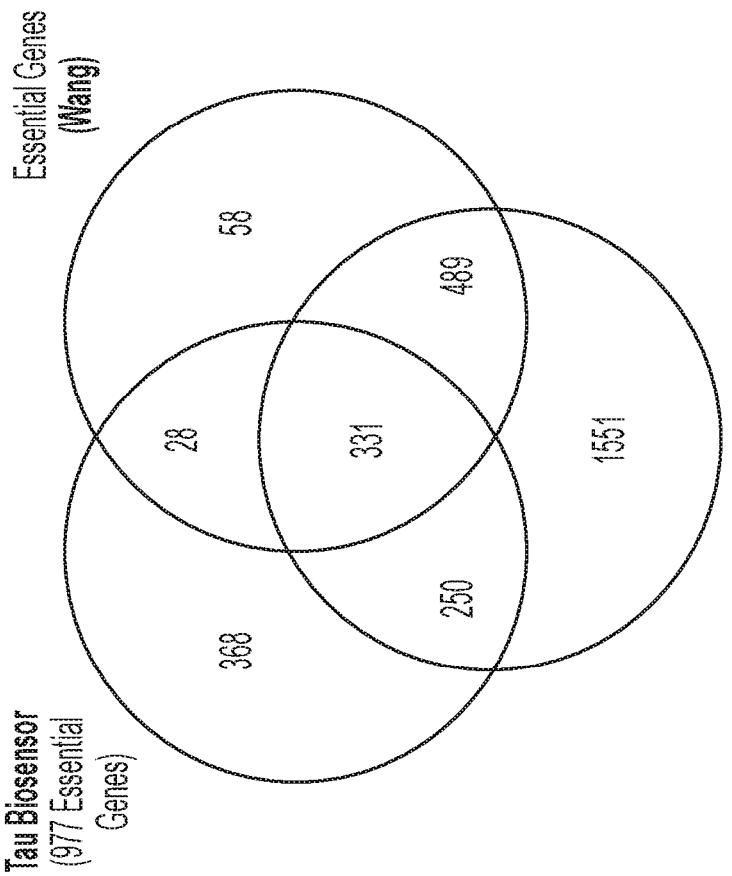
Tau Biosensor
(977 Essential
Genes)
Essential Genes
(Wang)
Essential Genes
(Tzelepis)
368
28
331
58
489
250
1551

FIG. 13

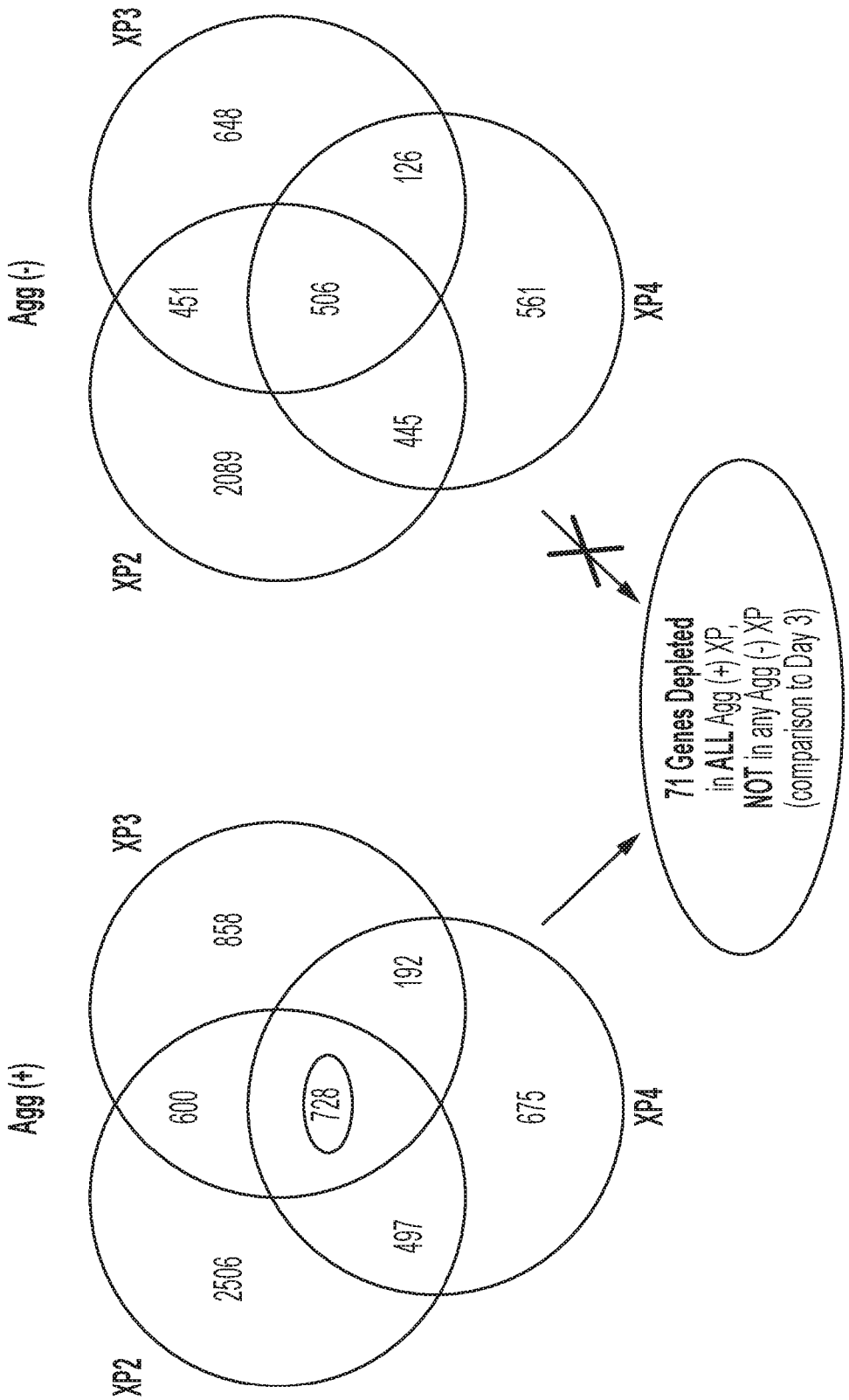
Agg (-)
XP3
XP2
XP4
648
126
451
506
561
2089
445
Agg (+)
XP3
XP2
XP4
858
192
600
728
675
2506
497
71 Genes Depleted
in ALL Agg (+) XP,
NOT in any Agg (-) XP
(comparison to Day 3)

FIG. 14

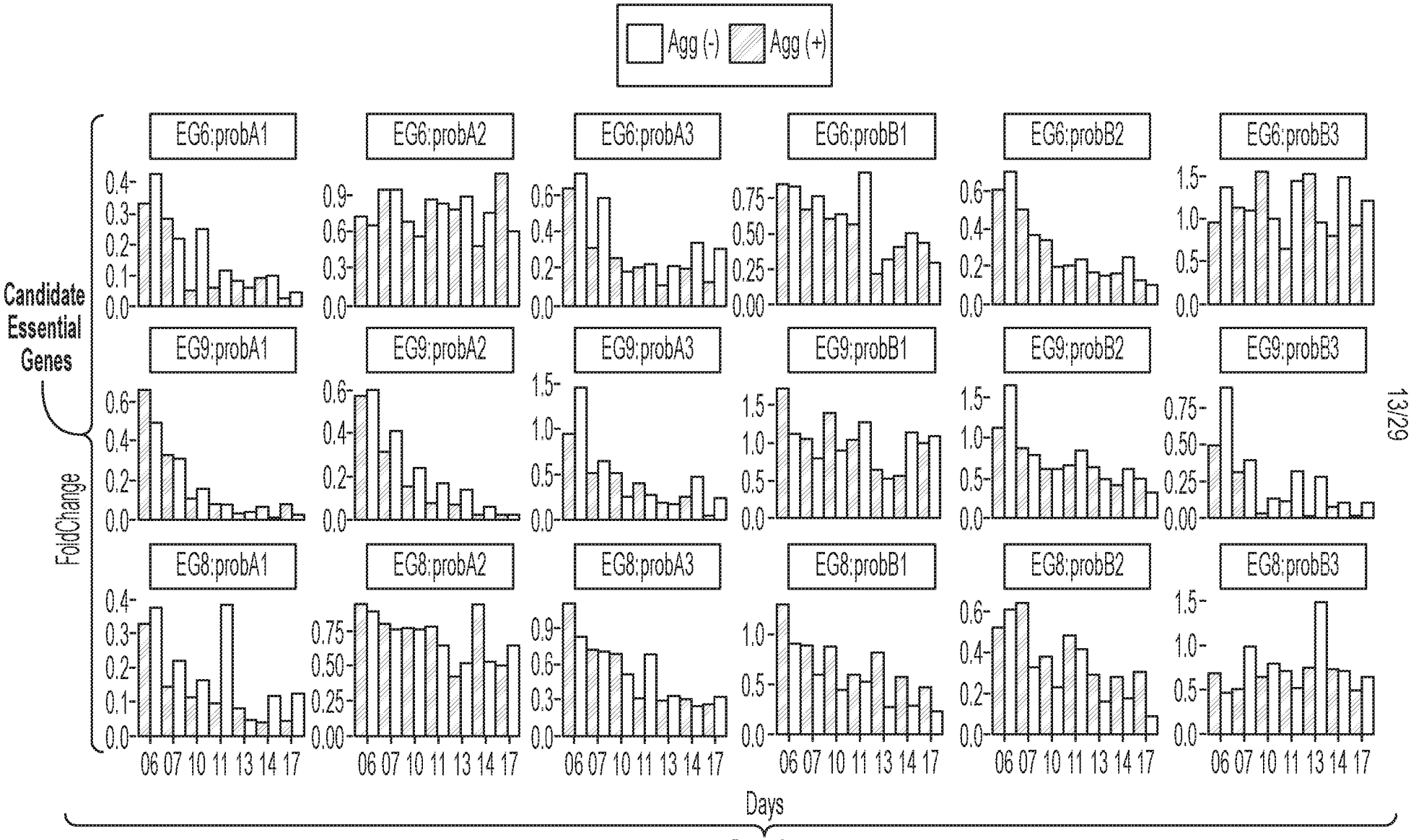
Agg (-)
Agg (+)
Candidate Essential Genes
FoldChange
EG6:probA1
EG6:probA2
EG6:probA3
EG6:probB1
EG6:probB2
EG6:probB3
EG9:probA1
EG9:probA2
EG9:probA3
EG9:probB1
EG9:probB2
EG9:probB3
EG8:probA1
EG8:probA2
EG8:probA3
EG8:probB1
EG8:probB2
EG8:probB3
06 07 10 11 13 14 17
Days

FIG. 15A

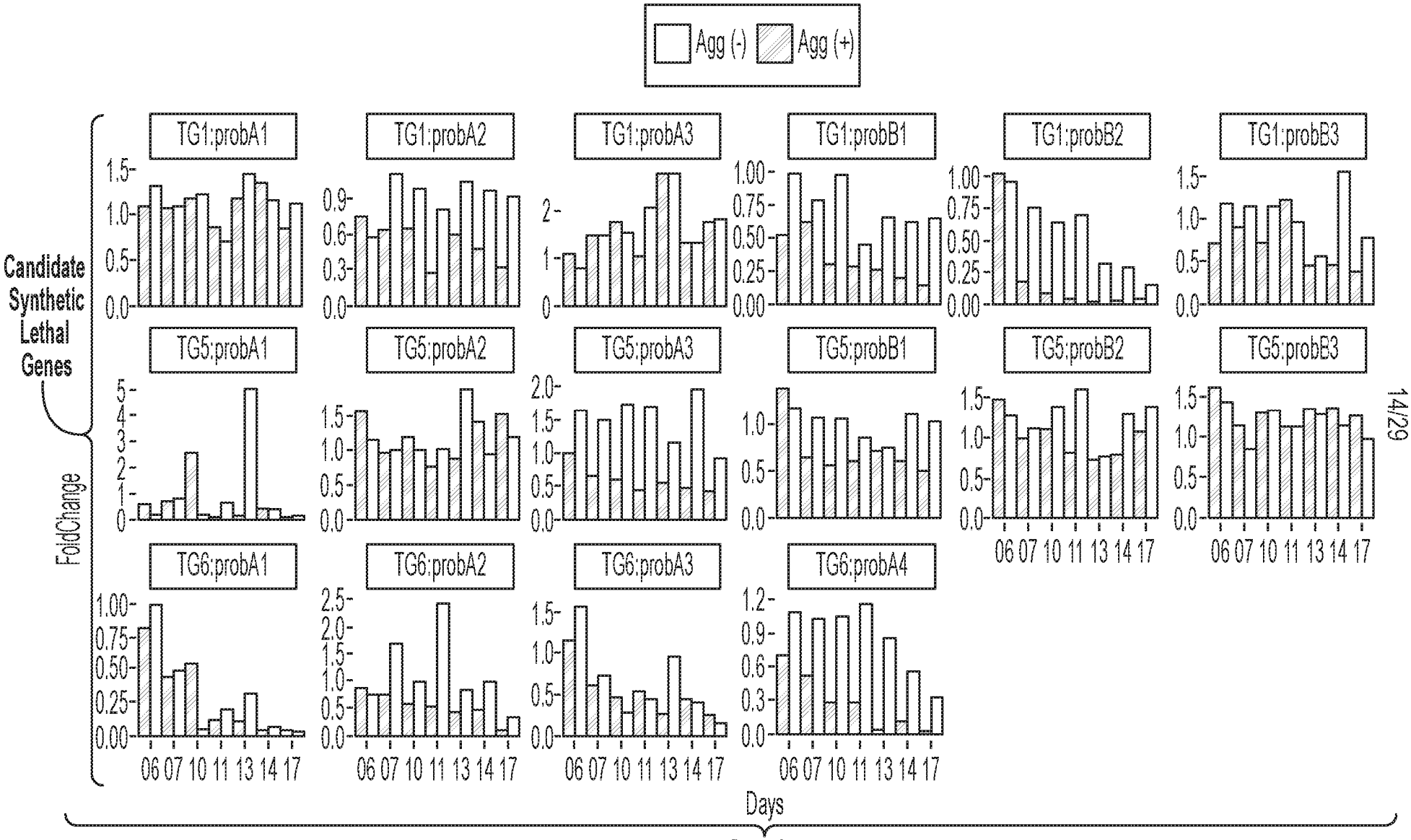
Agg (-)
Agg (+)
Candidate Synthetic Lethal Genes
FoldChange
TG1:probA1
TG1:probA2
TG1:probA3
TG1:probB1
TG1:probB2
TG1:probB3
TG5:probA1
TG5:probA2
TG5:probA3
TG5:probB1
TG5:probB2
TG5:probB3
TG6:probA1
TG6:probA2
TG6:probA3
TG6:probA4
06 07 10 11 13 14 17
Days
14/29

FIG. 15B

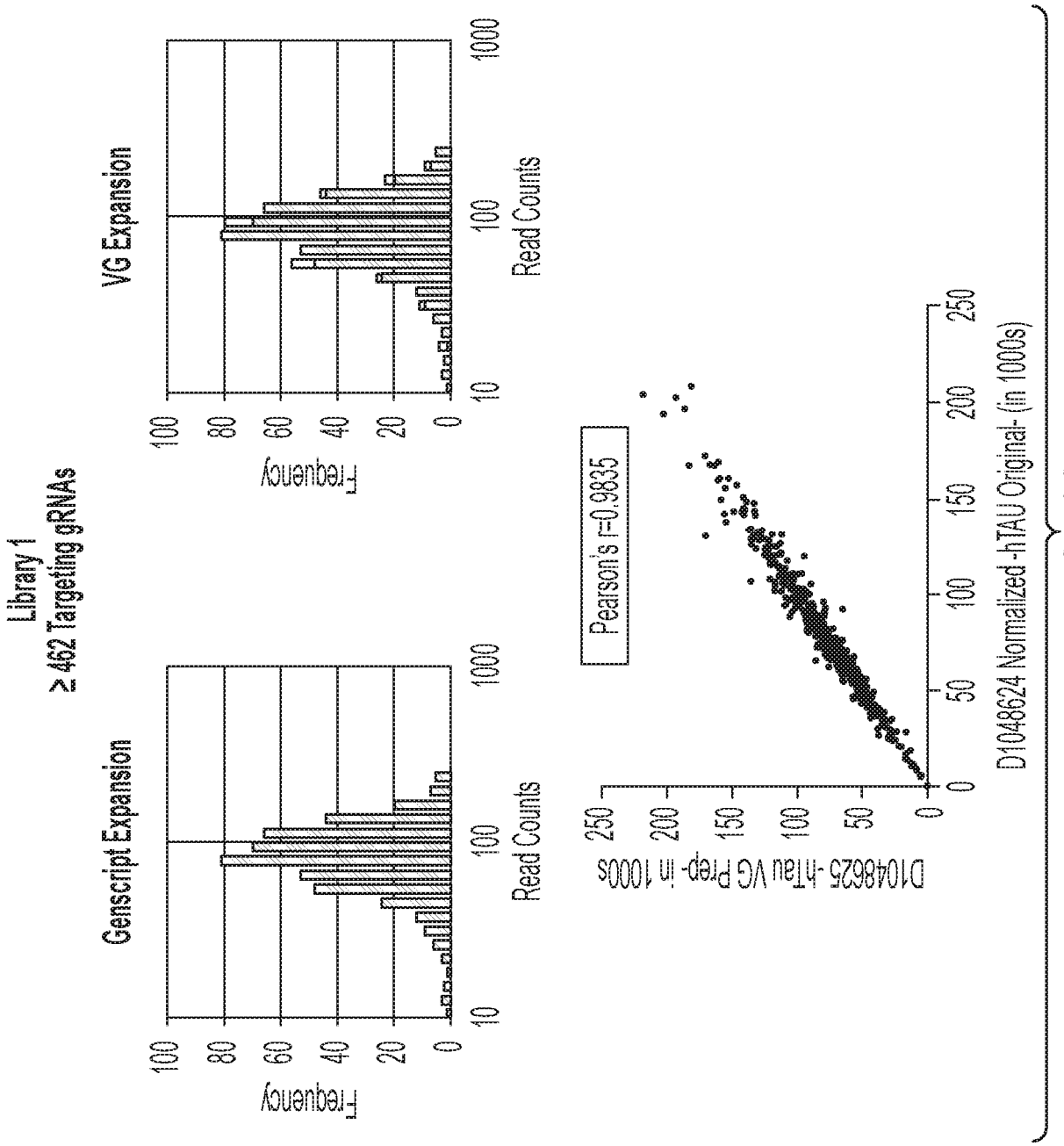
Library 1
≥ 462 Targeting gRNAs
Genscript Expansion
VG Expansion
Frequency
Read Counts
Pearson's r=0.9835
D1048625 -hTau VG Prep- in 1000s
D1048624 Normalized -hTAU Original- (in 1000s)

FIG. 16

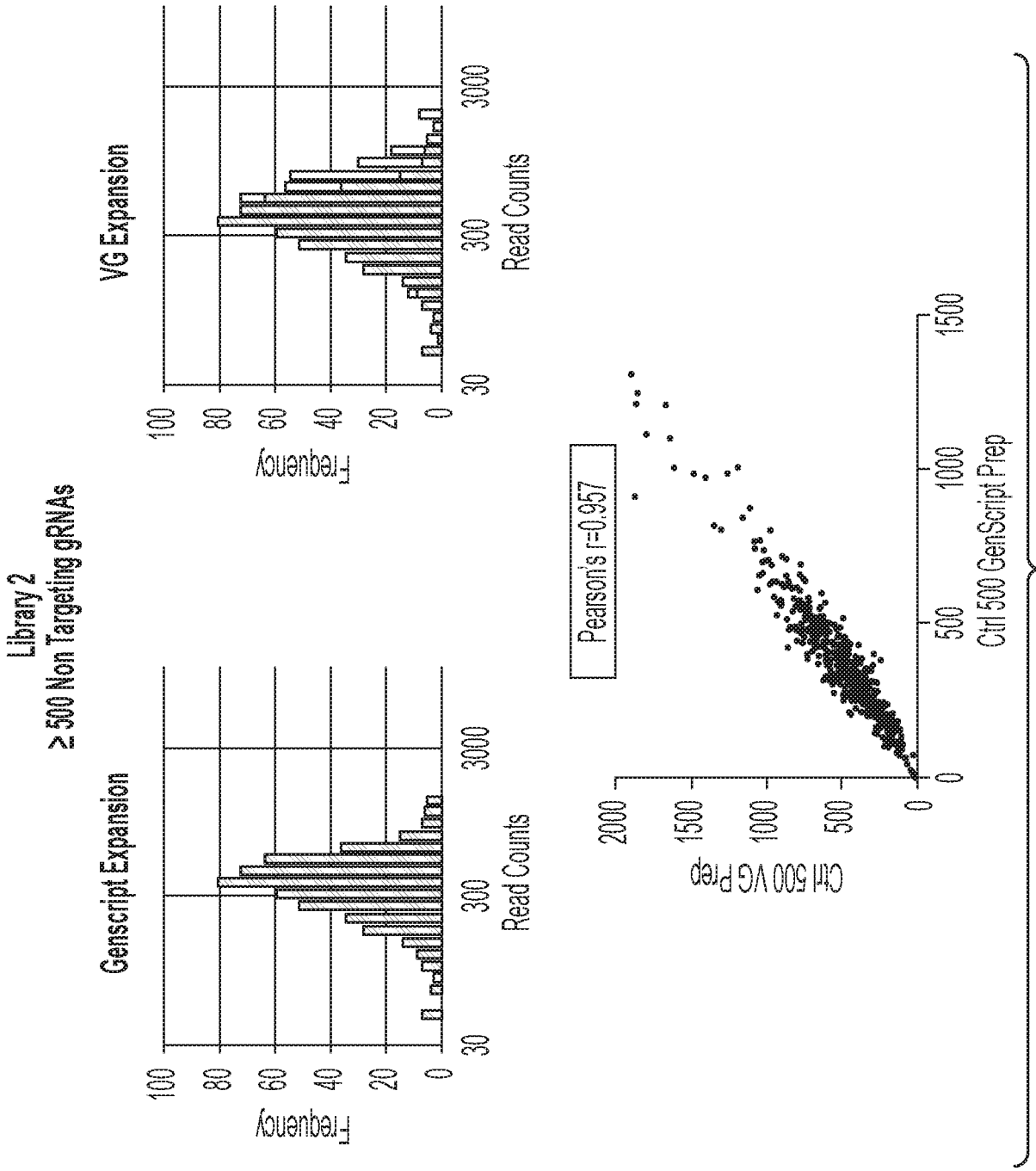
Library 2
≥ 500 Non Targeting gRNAs
Genscript Expansion
VG Expansion
Frequency
Read Counts
0
20
40
60
80
100
30
300
3000
Pearson's r=0.957
Ctrl 500 VG Prep
Ctrl 500 GenScript Prep
0
500
1000
1500
2000

FIG. 17

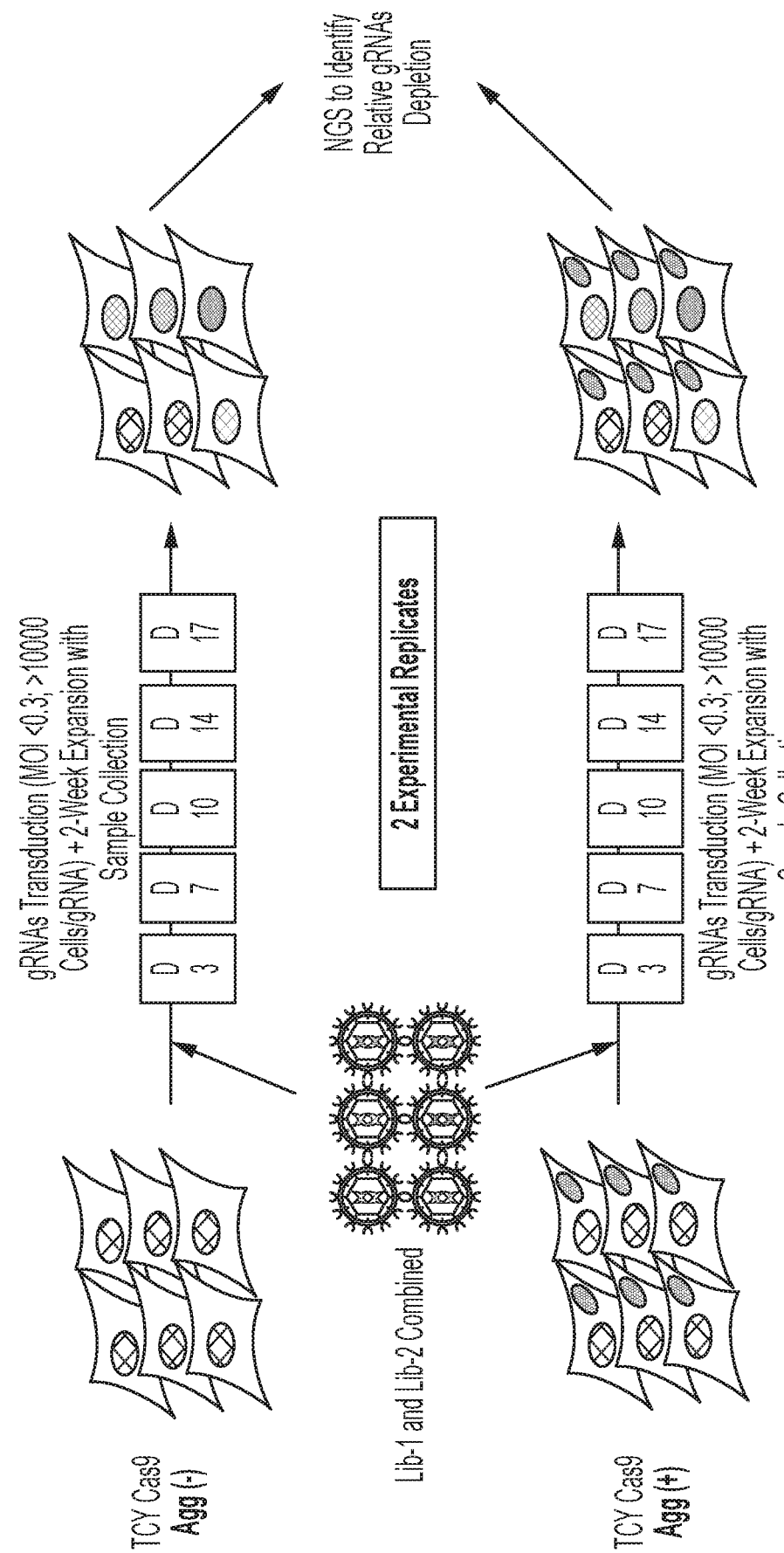
TCY Cas9
Agg (-)
Lib-1 and Lib-2 Combined
TCY Cas9
Agg (+)
gRNAs Transduction (MOI <0.3; >10000 Cells/gRNA) + 2-Week Expansion with Sample Collection
D 3
D 7
D 10
D 14
D 17
2 Experimental Replicates
NGS to Identify Relative gRNAs Depletion

FIG. 18

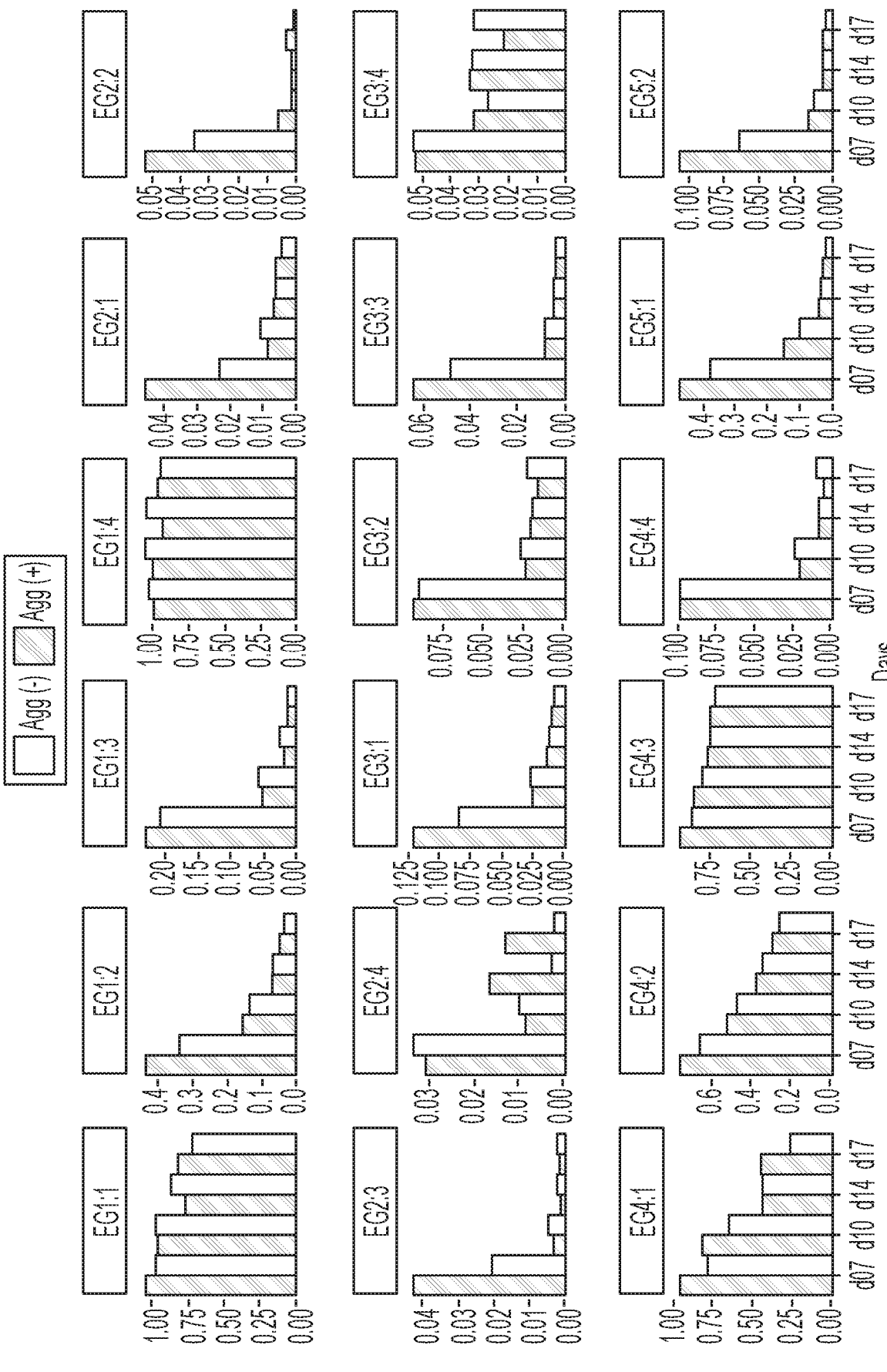
Agg (-)
Agg (+)
EG1:1
EG1:2
EG1:3
EG1:4
EG2:1
EG2:2
EG2:3
EG2:4
EG3:1
EG3:2
EG3:3
EG3:4
EG4:1
EG4:2
EG4:3
EG4:4
EG5:1
EG5:2
foldchange
Days
d07 d10 d14 d17

FIG. 19A

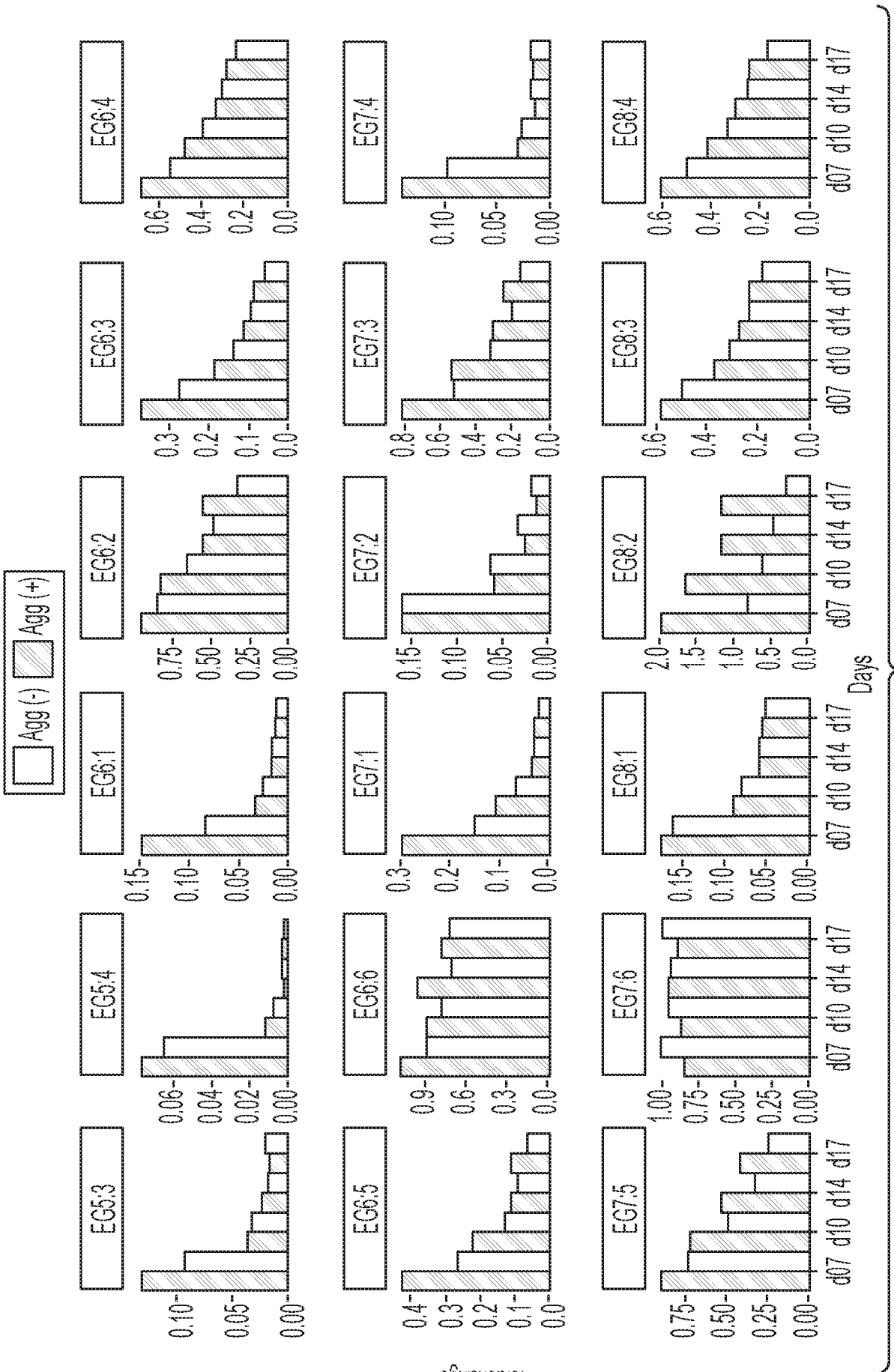
Agg (-)
Agg (+)
EG5:3
EG5:4
EG6:1
EG6:2
EG6:3
EG6:4
EG6:5
EG6:6
EG7:1
EG7:2
EG7:3
EG7:4
EG7:5
EG7:6
EG8:1
EG8:2
EG8:3
EG8:4
foldchange
Days
d07 d10 d14 d17

FIG. 19B

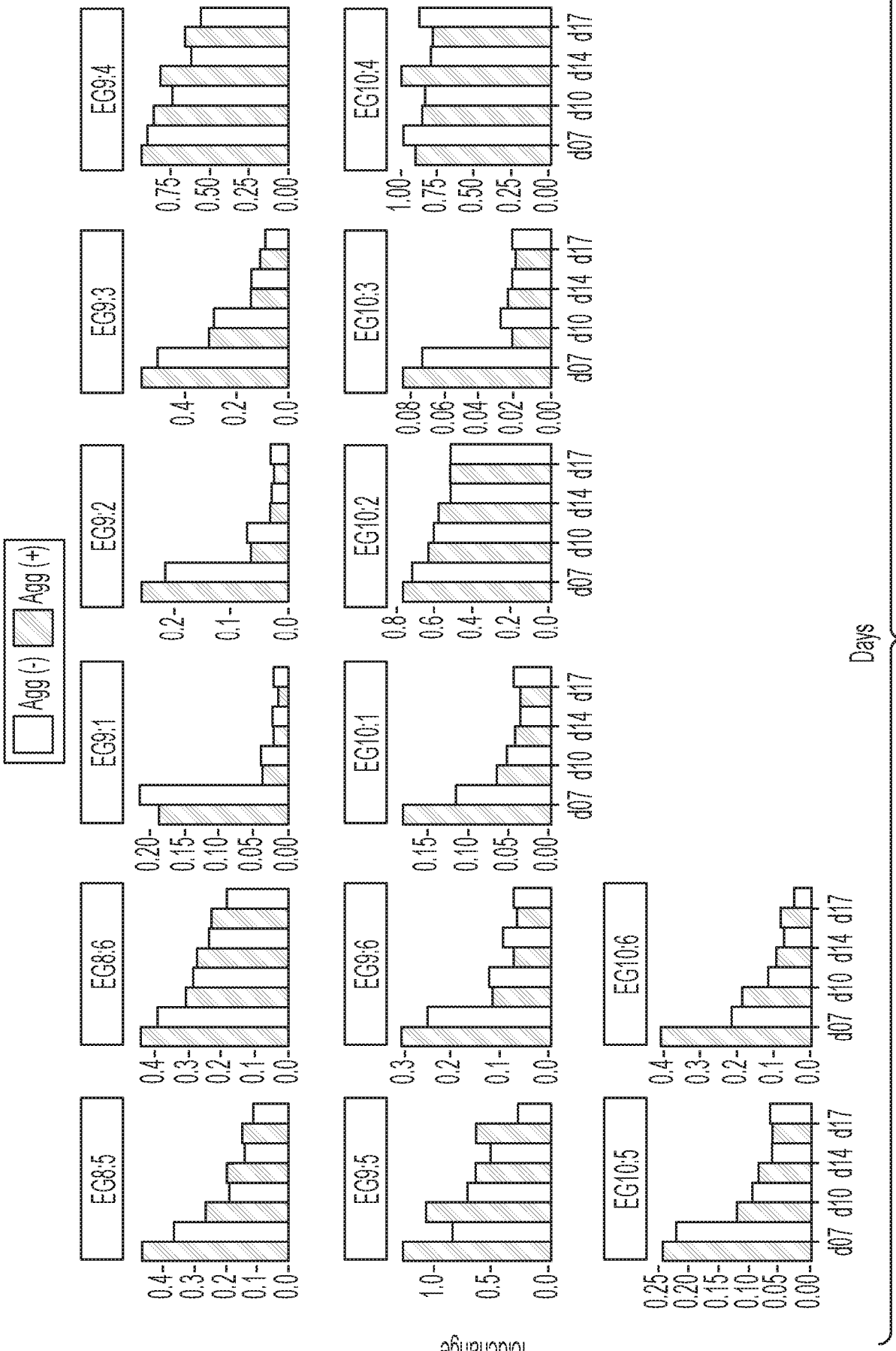
Agg (-)
Agg (+)
EG8:5
EG8:6
EG9:1
EG9:2
EG9:3
EG9:4
EG9:5
EG9:6
EG10:1
EG10:2
EG10:3
EG10:4
EG10:5
EG10:6
foldchange
Days
d07 d10 d14 d17

FIG. 19C

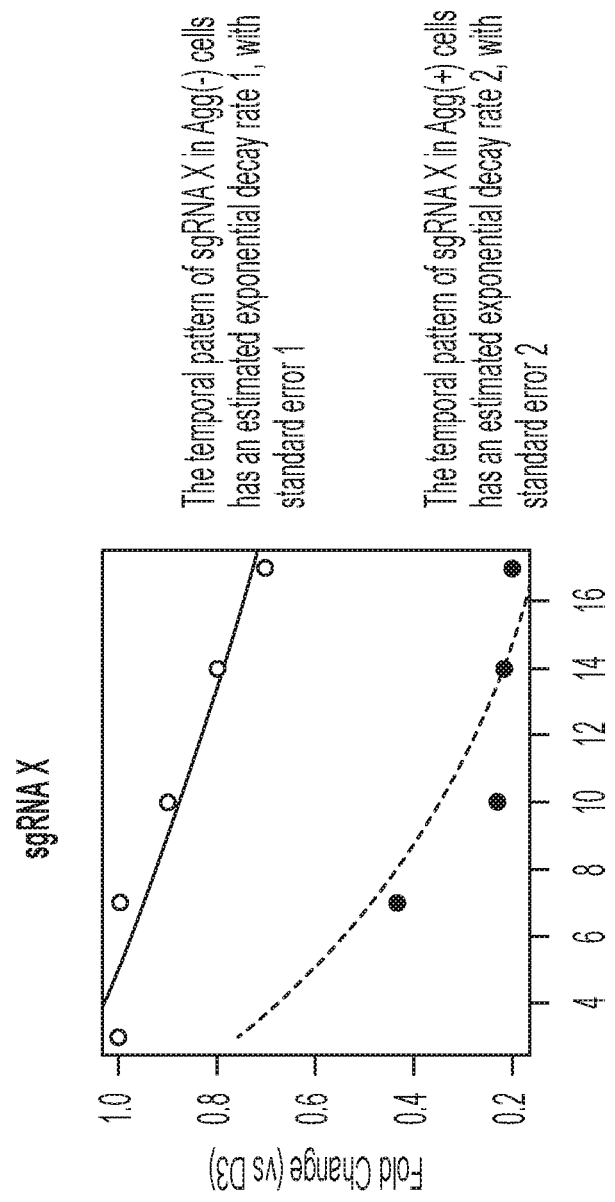
sgRNA X
Fold Change (vs D3)
1.0
0.8
0.6
0.4
0.2
4
6
8
10
12
14
16
Time (Day)
The temporal pattern of sgRNA X in Agg(-) cells has an estimated exponential decay rate 1, with standard error 1
The temporal pattern of sgRNA X in Agg(+) cells has an estimated exponential decay rate 2, with standard error 2

FIG. 20

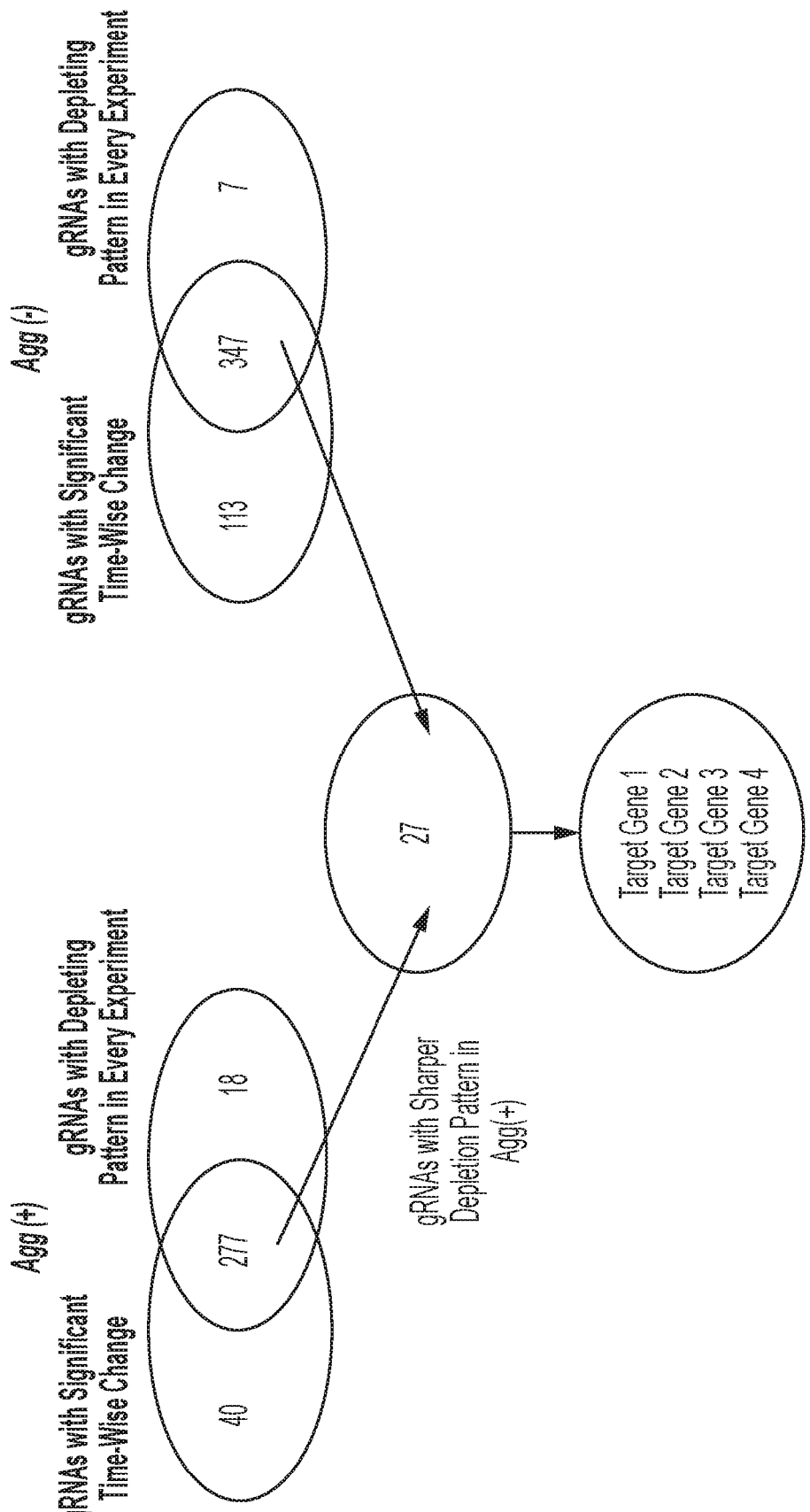
Agg (+)
gRNAs with Significant Time-Wise Change
40
277
Agg (+)
gRNAs with Depleting Pattern in Every Experiment
18
gRNAs with Sharper Depletion Pattern in Agg(+)
27
Agg (-)
gRNAs with Significant Time-Wise Change
113
347
Agg (-)
gRNAs with Depleting Pattern in Every Experiment
7
Target Gene 1
Target Gene 2
Target Gene 3
Target Gene 4

FIG. 21

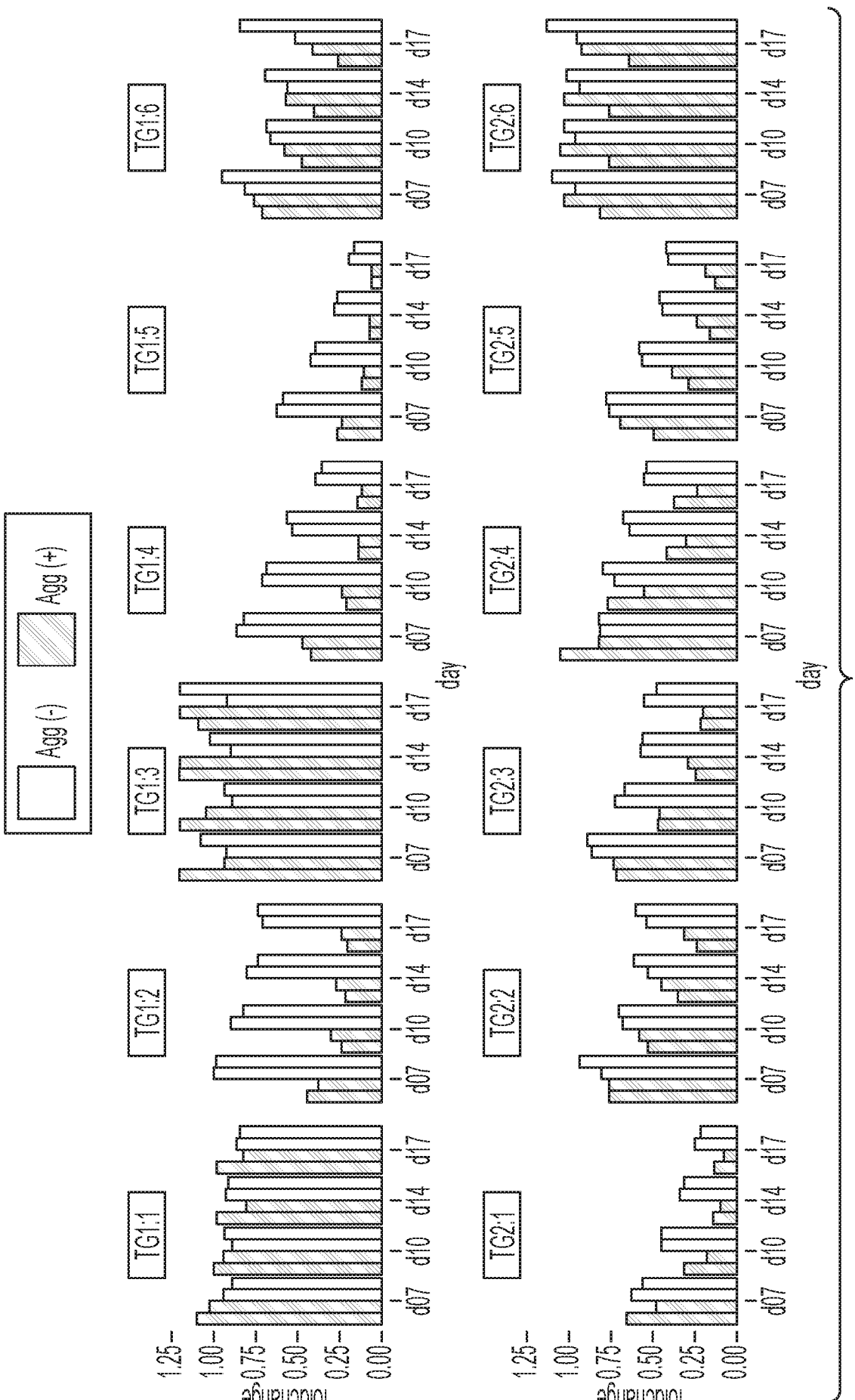
Agg (-)
Agg (+)
TG1:1
TG1:2
TG1:3
TG1:4
TG1:5
TG1:6
TG2:1
TG2:2
TG2:3
TG2:4
TG2:5
TG2:6
foldchange
1.25
1.00
0.75
0.50
0.25
0.00
d07
d10
d14
d17
day

FIG. 22A

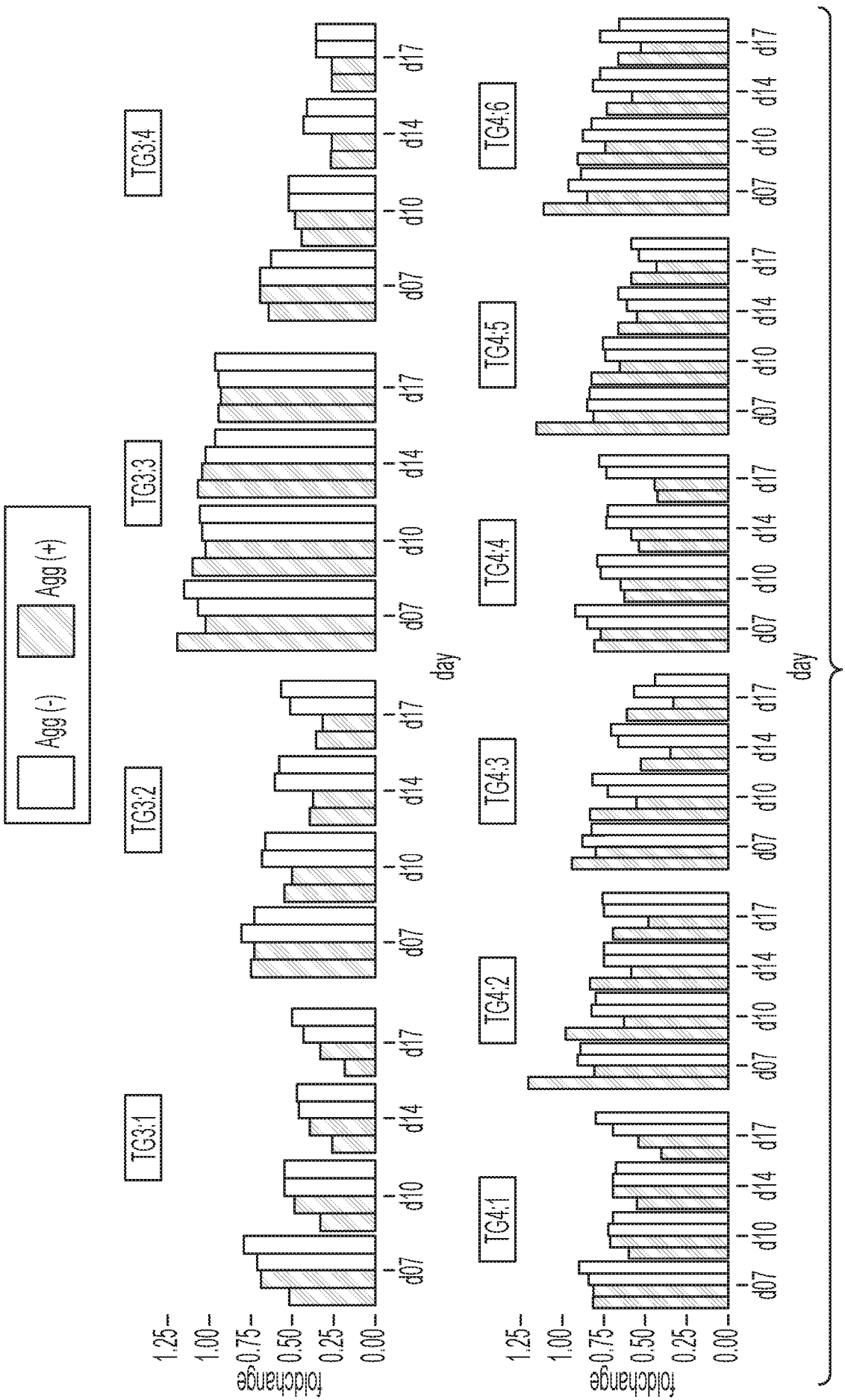
Agg (-)
Agg (+)
TG3:1
TG3:2
TG3:3
TG3:4
TG4:1
TG4:2
TG4:3
TG4:4
TG4:5
TG4:6
foldchange
1.25
1.00
0.75
0.50
0.25
0.00
d07
d10
d14
d17
day

FIG. 22B

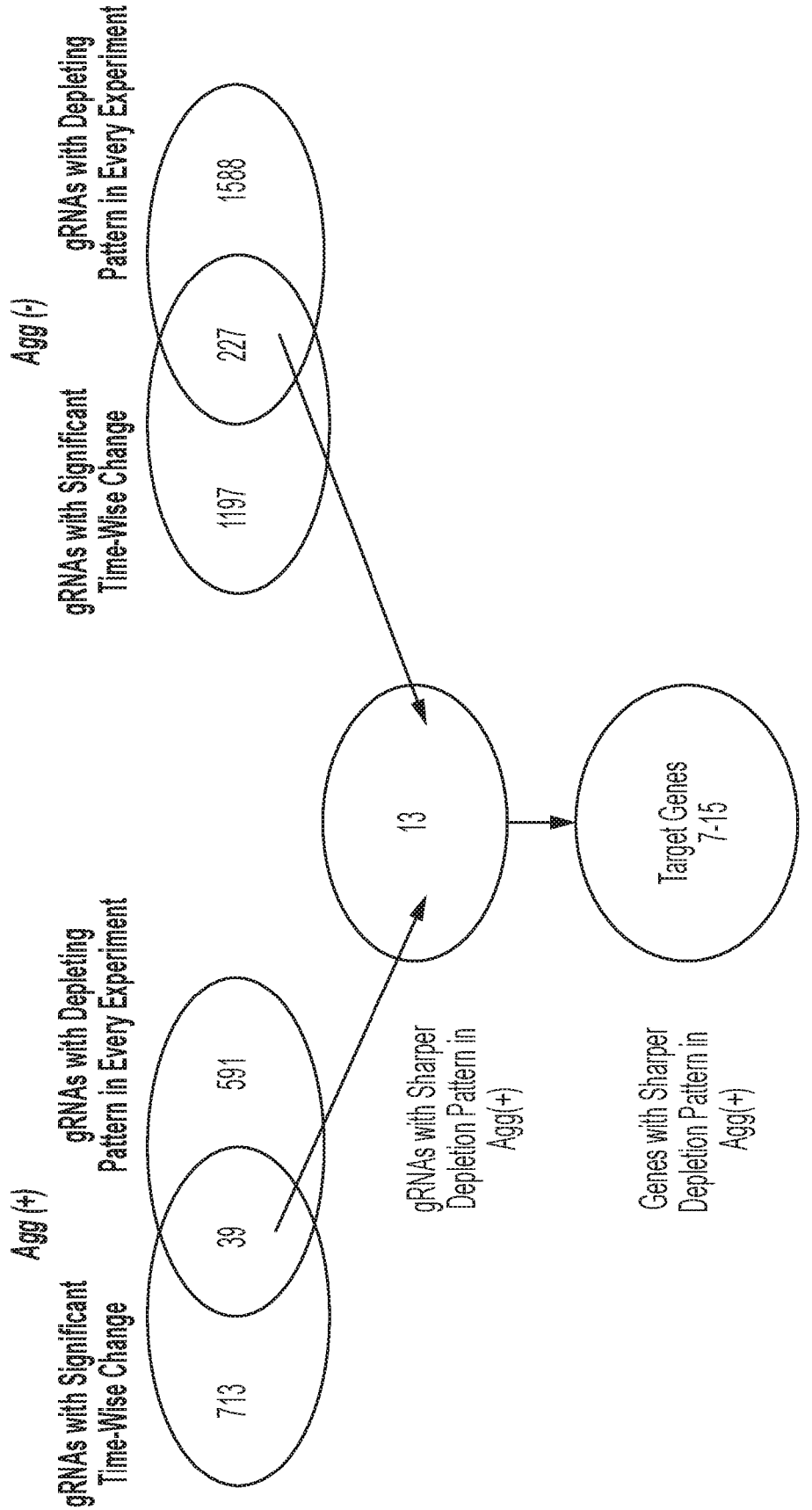
Agg (+)
gRNAs with Significant Time-Wise Change
713
39
gRNAs with Depleting Pattern in Every Experiment
591
Agg (-)
gRNAs with Significant Time-Wise Change
1197
227
gRNAs with Depleting Pattern in Every Experiment
1588
13
gRNAs with Sharper Depletion Pattern in Agg(+)
Target Genes 7-15
Genes with Sharper Depletion Pattern in Agg(+)

FIG. 23

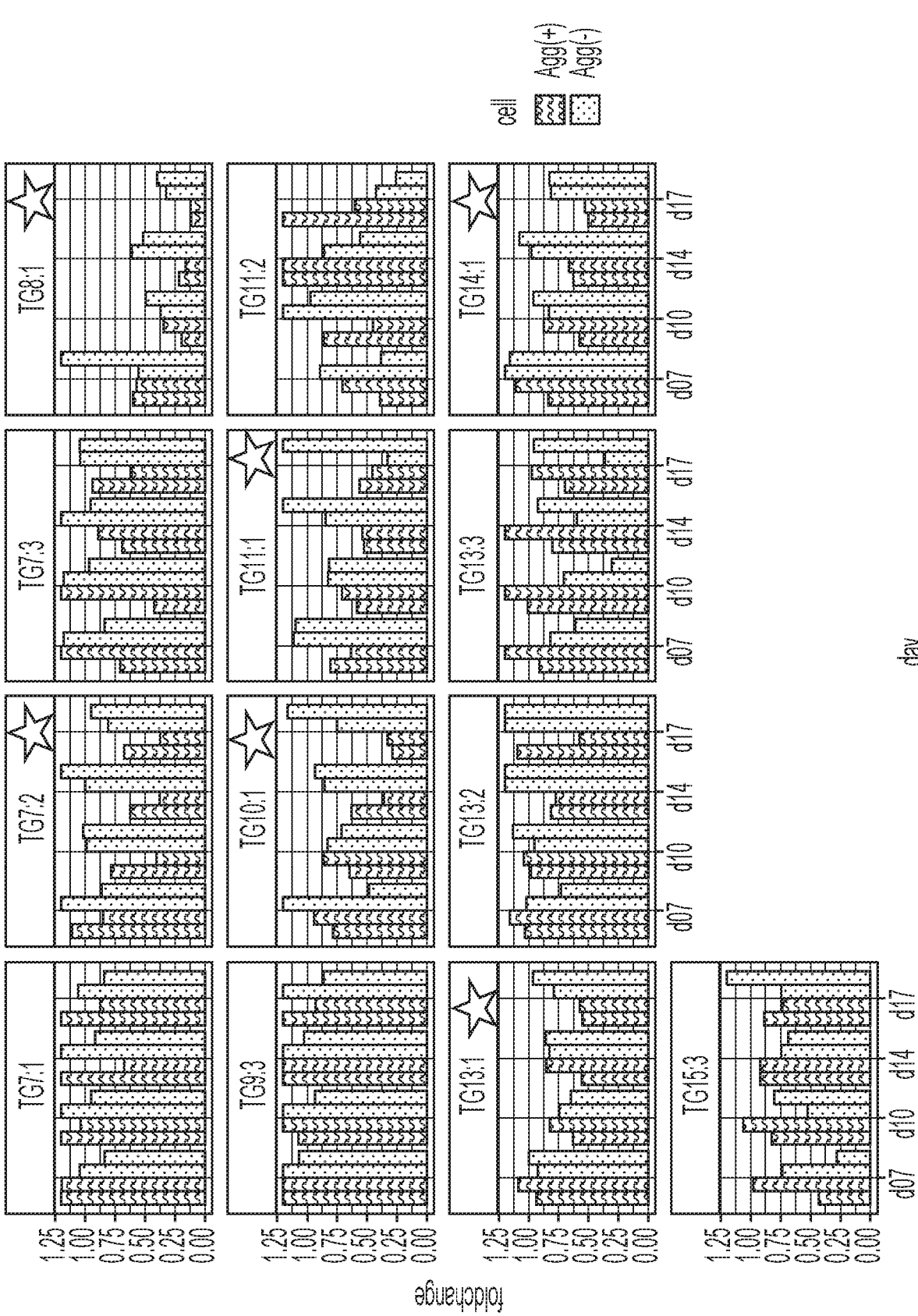
TG7:1
TG7:2
TG7:3
TG8:1
TG9:3
TG10:1
TG11:1
TG11:2
TG13:1
TG13:2
TG13:3
TG14:1
TG15:3
1.25
1.00
0.75
0.50
0.25
0.00
foldchange
d07
d10
d14
d17
day
cell
Agg(+)
Agg(-)

FIG. 24A

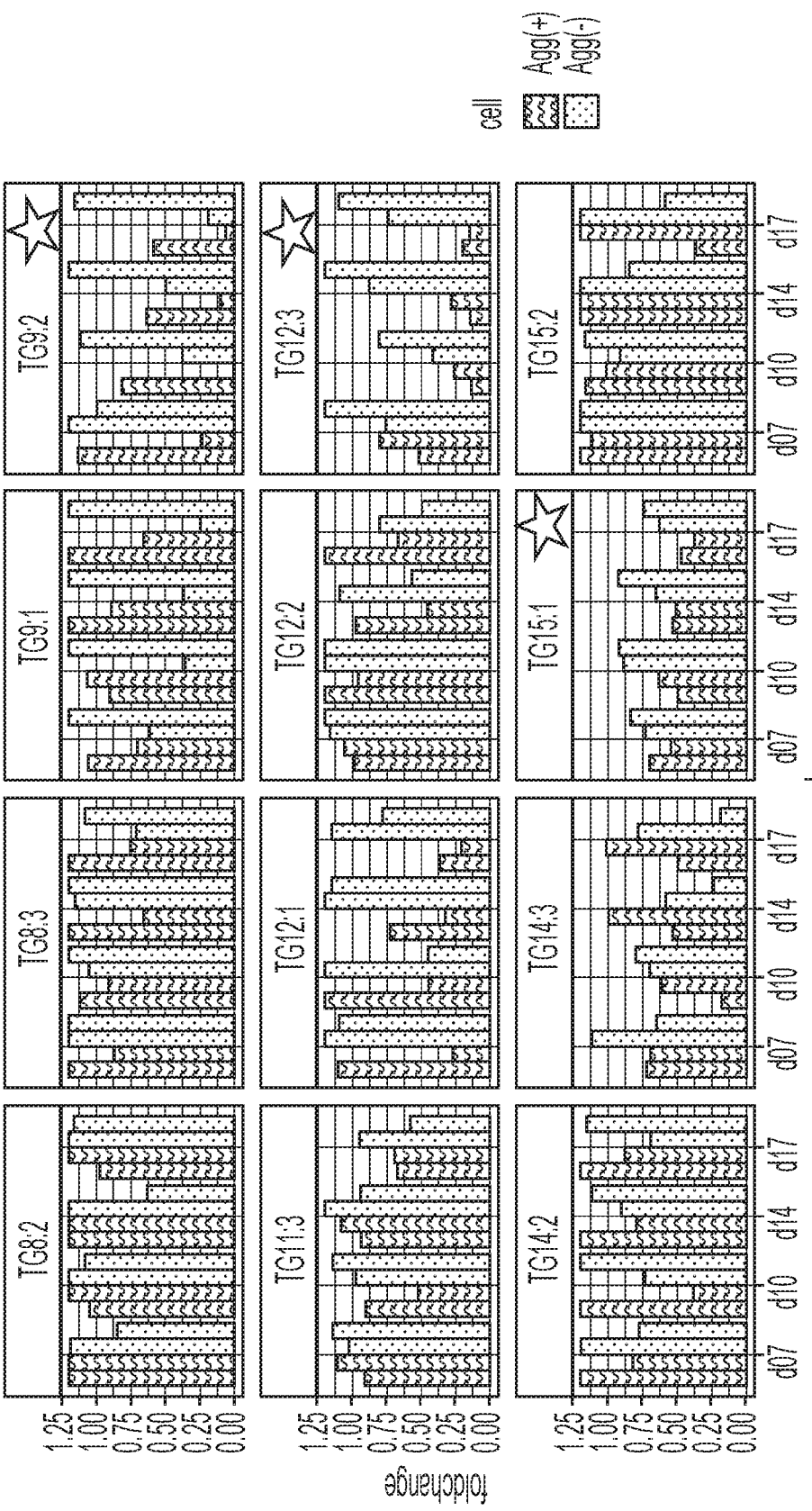
TG8:2
TG8:3
TG9:1
TG9:2
TG11:3
TG12:1
TG12:2
TG12:3
TG14:2
TG14:3
TG15:1
TG15:2
foldchange
1.25
1.00
0.75
0.50
0.25
0.00
d07
d10
d14
d17
day
cell
Agg(+)
Agg(-)

FIG. 24B

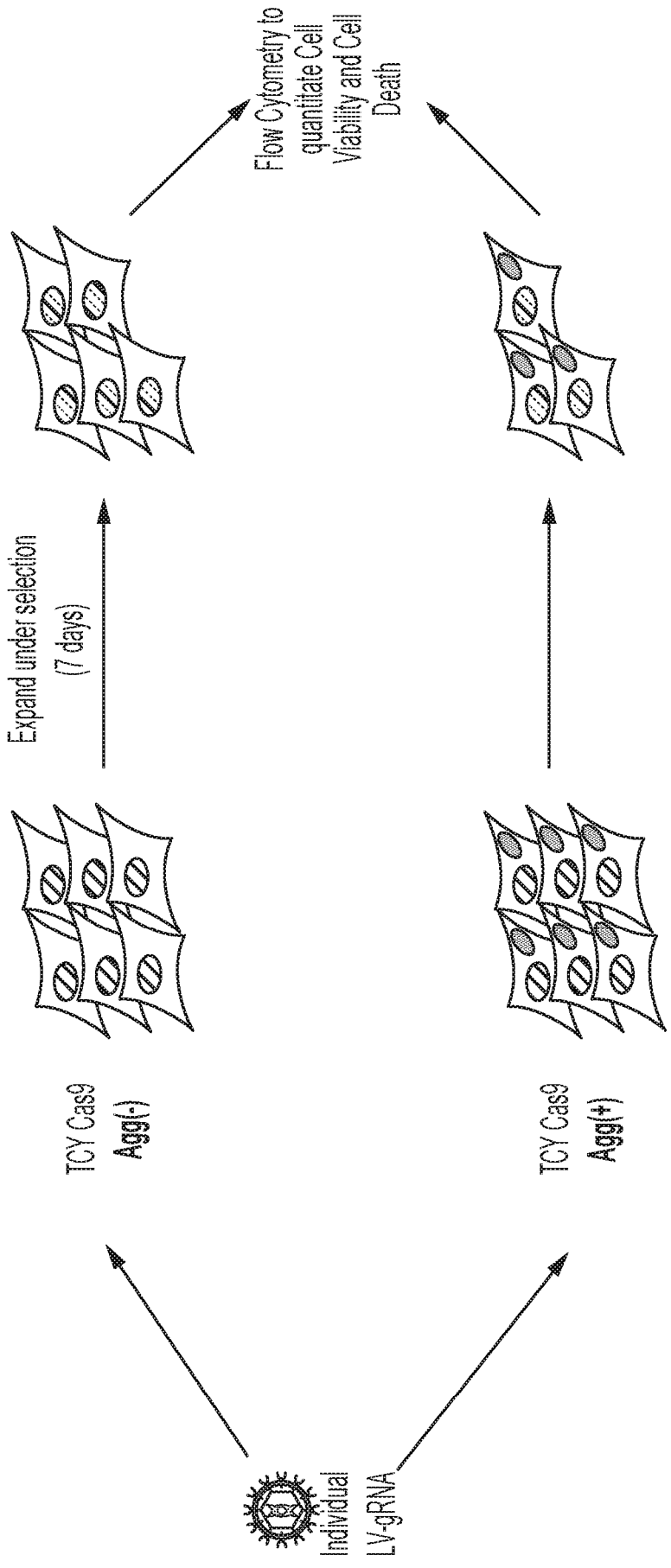
Individual LV-gRNA
TCY Cas9 Agg(-)
TCY Cas9 Agg(+)
Expand under selection (7 days)
Flow Cytometry to quantitate Cell Viability and Cell Death

FIG. 25

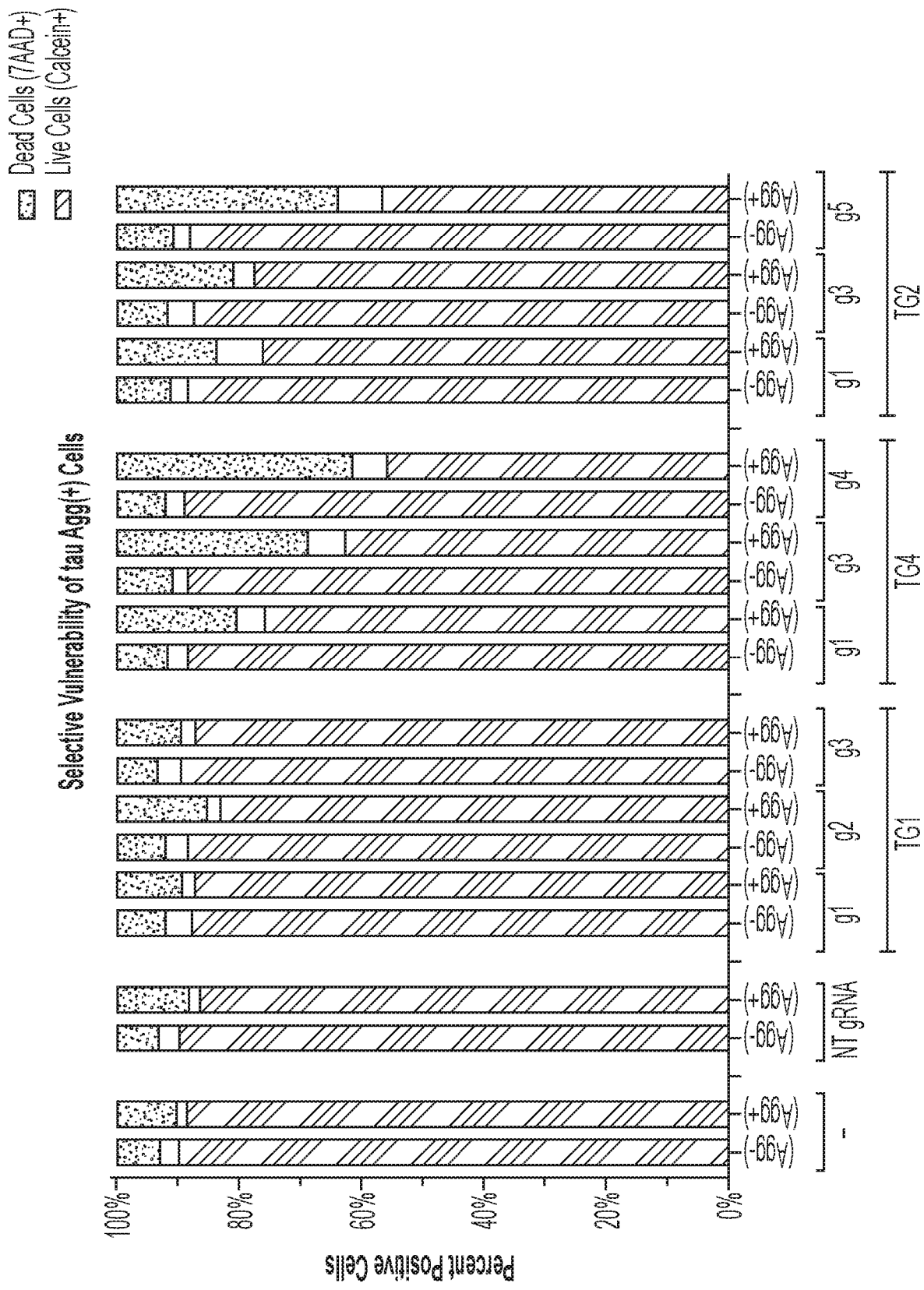
Dead Cells (7AAD+)
Live Cells (Calcein+)
Selective Vulnerability of tau Aggr(+) Cells
Percent Positive Cells
100%
80%
60%
40%
20%
0%
(Aβ99+)
(Aβ99-)
-
NT gRNA
g1
g2
g3
g4
g5
TG1
TG2
TG4

FIG. 26