FIG. 1A

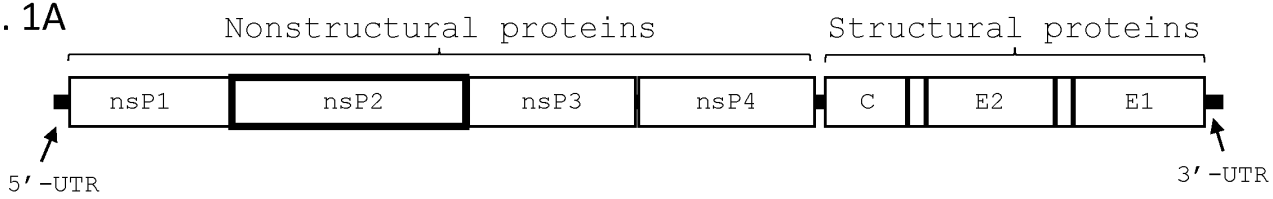


FIG. 1B

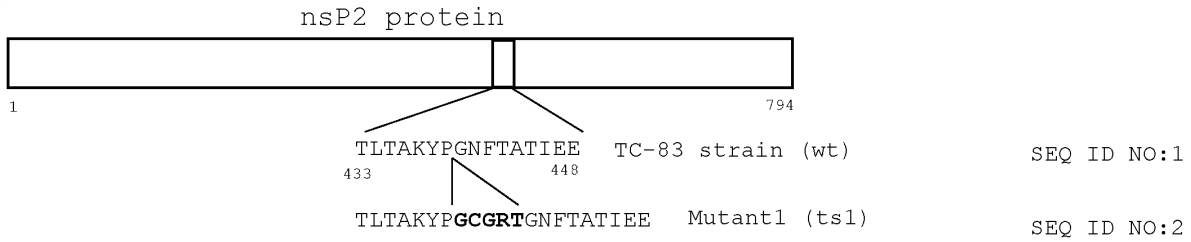


FIG. 1C

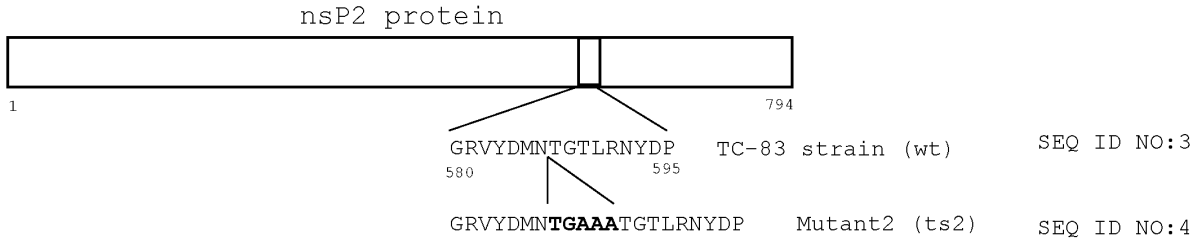
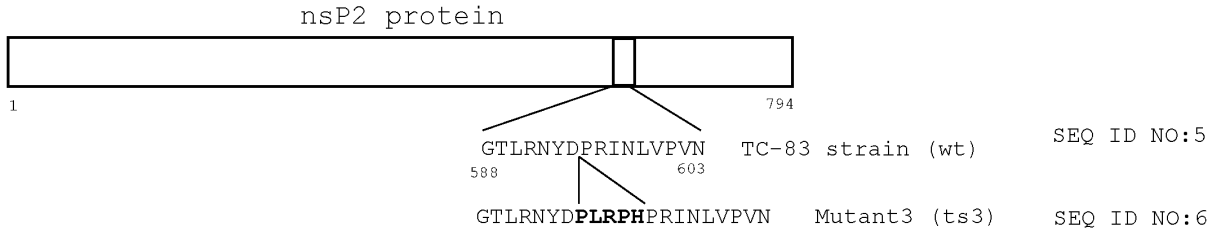


FIG. 1D



TC-83 (wt) ACACTGACTGCCAAGTACCCTGGGAATTTCACTGCCACG

SEQ ID NO:7

Mutant 1 (ts1) ACACTGACTGCCAAGTACC**CTGGGTGCGGCCGGA**CTGGGAATTTCACTGCCACG

SEQ ID NO:8

SEQ ID NO:13 TLTAKYPGNFTAT

SEQ ID NO:14 TLTAKYP**GCGRT**GNFTAT

FIG. 2A

TC-83 (wt) GGAAGAGTCTATGACATGAACACTGGTACACTGCGCAAT

SEQ ID NO:9

Mutant 2 (ts2) GGAAGAGTCTATGACATGAAC**ACTGGTGCCGCCGCA**ACTGGTACACTGCGCAAT

SEQ ID NO:10

SEQ ID NO:15 GRVYDMNTGTLRN

SEQ ID NO:16 GRVYDMN**TGAAA**TGTLRN

FIG. 2B

TC-83 (wt) GGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTA

SEQ ID NO:11

Mutant 3 (ts3) GGTACACTGCGCAATTATG**ATCCGCTGCGGCCCCC**ATCCGCGCATAAACCTAGTA

SEQ ID NO:12

SEQ ID NO:17 GTLRNYDPRINLV

SEQ ID NO:18 GTLRNYDP**LRPHP**RINLV

FIG. 2C

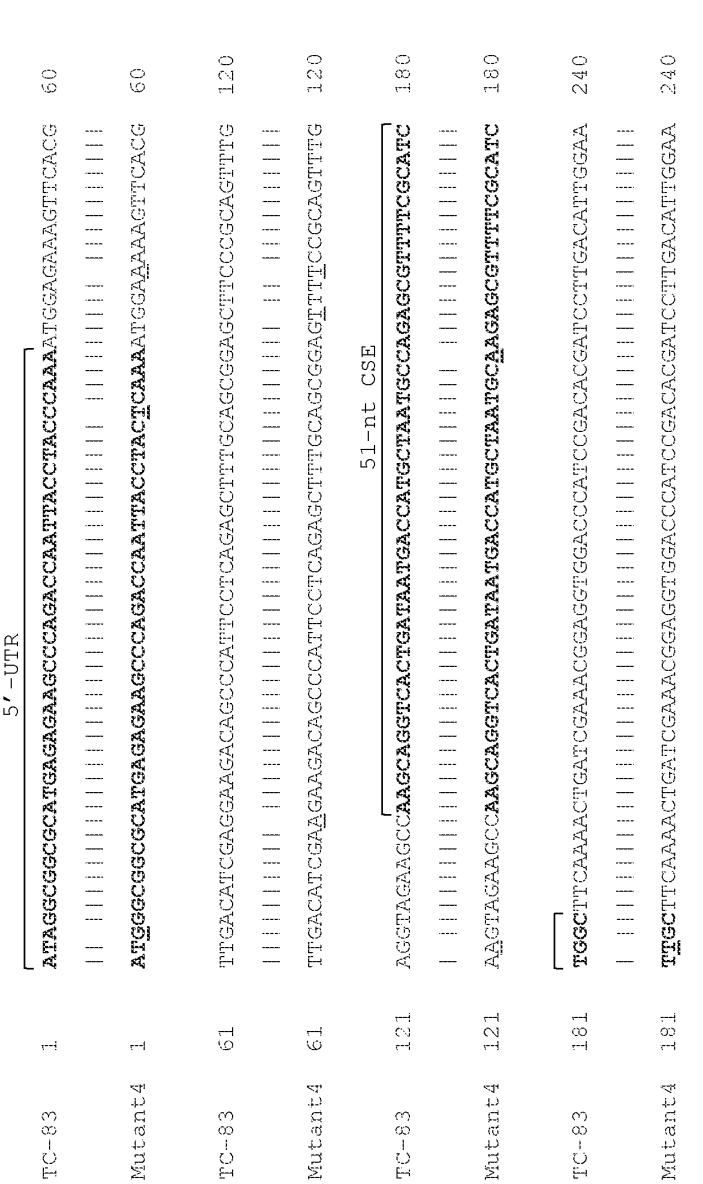

5′-UTR
TC-83 1 ATAGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAGTTCACG 60
Mutant4 1 ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACTCAAAATGGAAAAACTTCACG 60
TC-83 61 TTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTTCCCGCAGTTTG 120
Mutant4 61 TTGACATCGAAGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGTTTTCCGCAGTTTG 120
51-nt CSE
TC-83 121 AGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTTCGCATC 180
Mutant4 121 AAGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCAAGAGCGTTTTTCGCATC 180
TC-83 181 TGGCTTCAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAA 240
Mutant4 181 TTGCTTCAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAA 240

FIG. 3

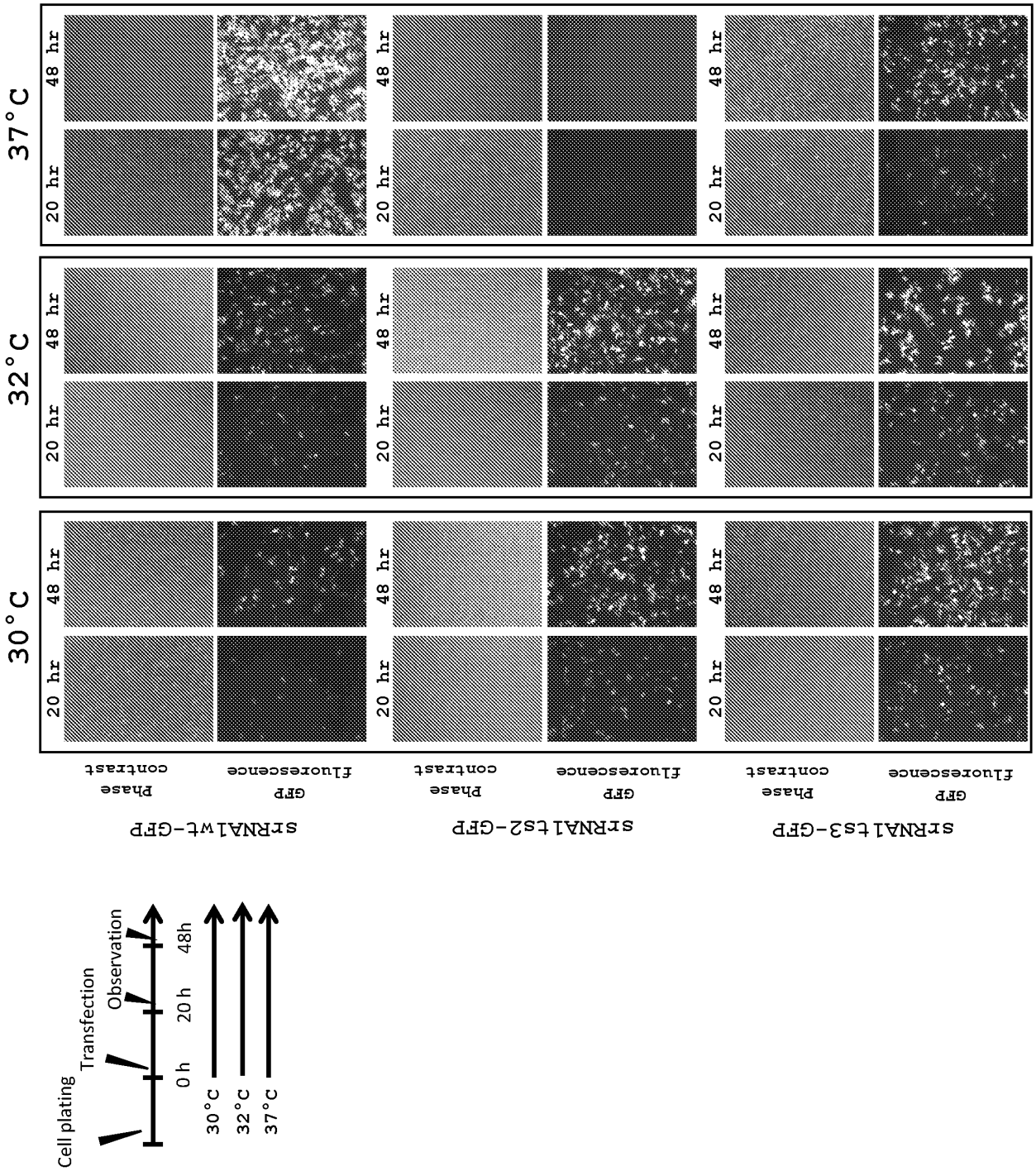
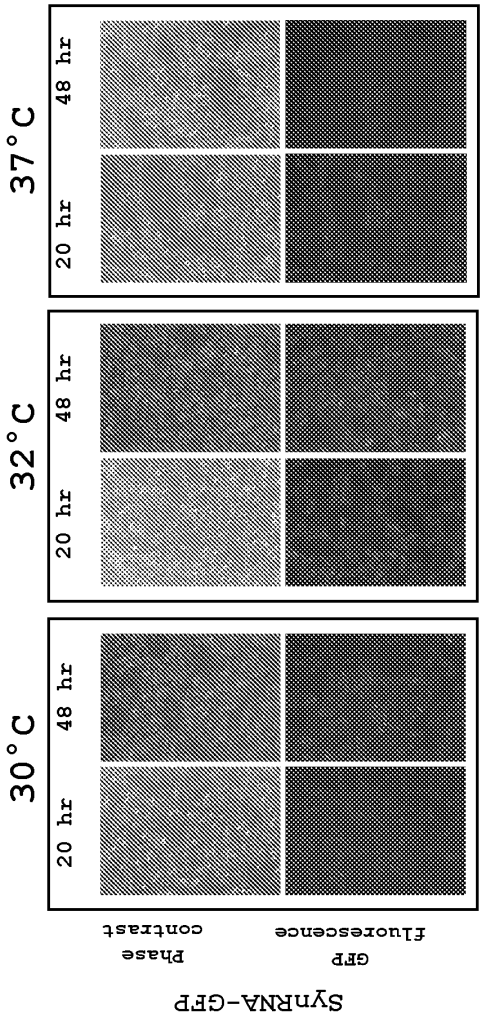

37°C
32°C
30°C
20 hr
48 hr
srRNA1wt-GFP
srRNA1ts2-GFP
srRNA1ts3-GFP
GFP fluorescence
Phase contrast
Cell plating
Transfection
Observation
0 h
20 h
48h
30°C
32°C
37°C

FIG. 4A


30°C
32°C
37°C
20 hr
48 hr
SynRNA-GFP
GFP
fluorescence
Phase
contrast

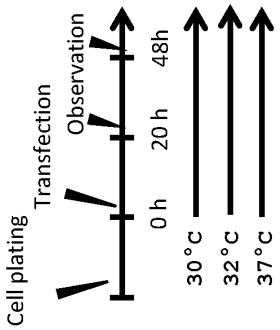

Cell plating
Transfection
Observation
0 h
20 h
48h
30°C
32°C
37°C

FIG. 4B

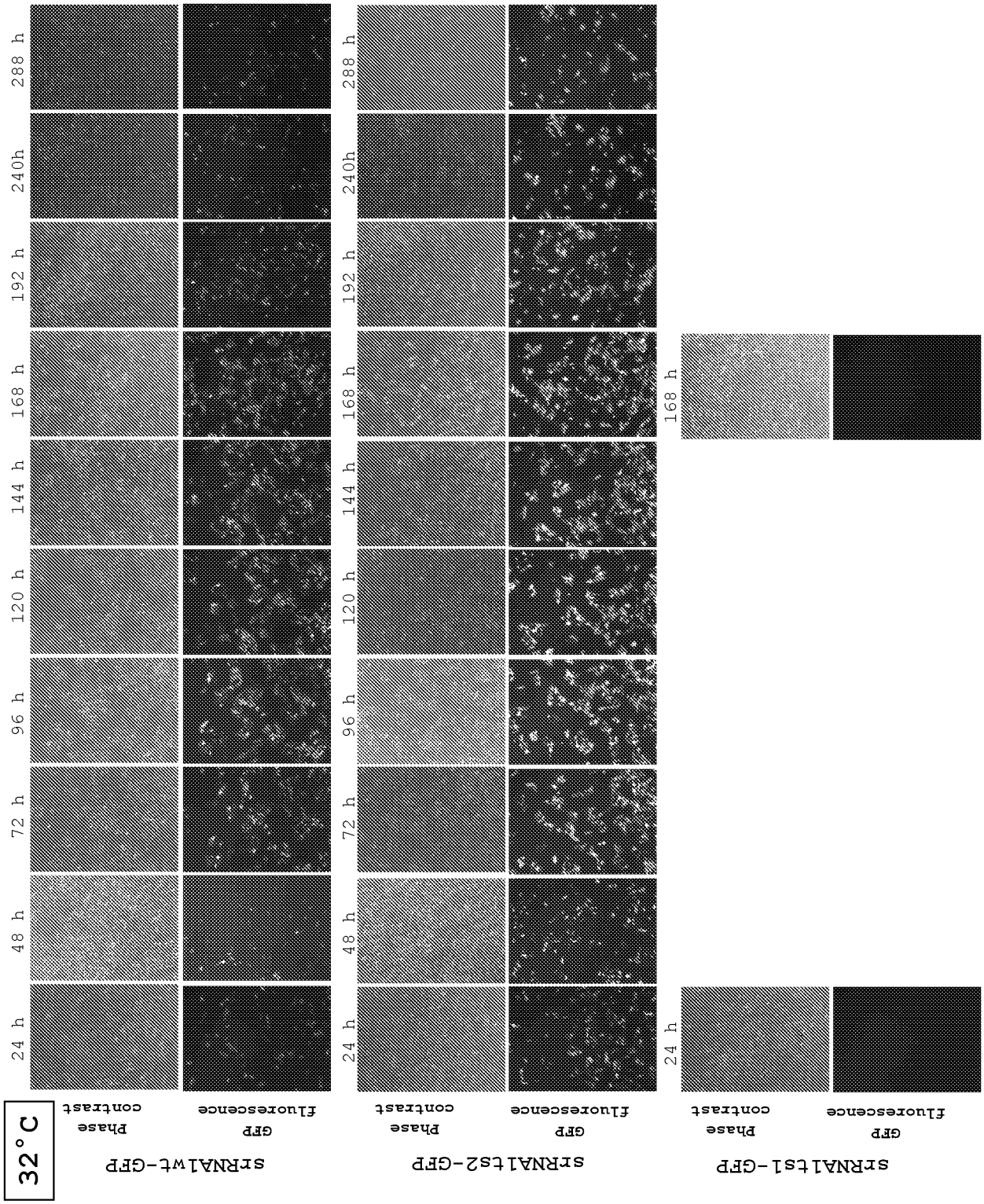

32°C
srRNA1wt-GFP
srRNA1ts2-GFP
srRNA1ts1-GFP
GFP fluorescence
Phase contrast
24 h
48 h
72 h
96 h
120 h
144 h
168 h
192 h
240h
288 h

FIG. 5

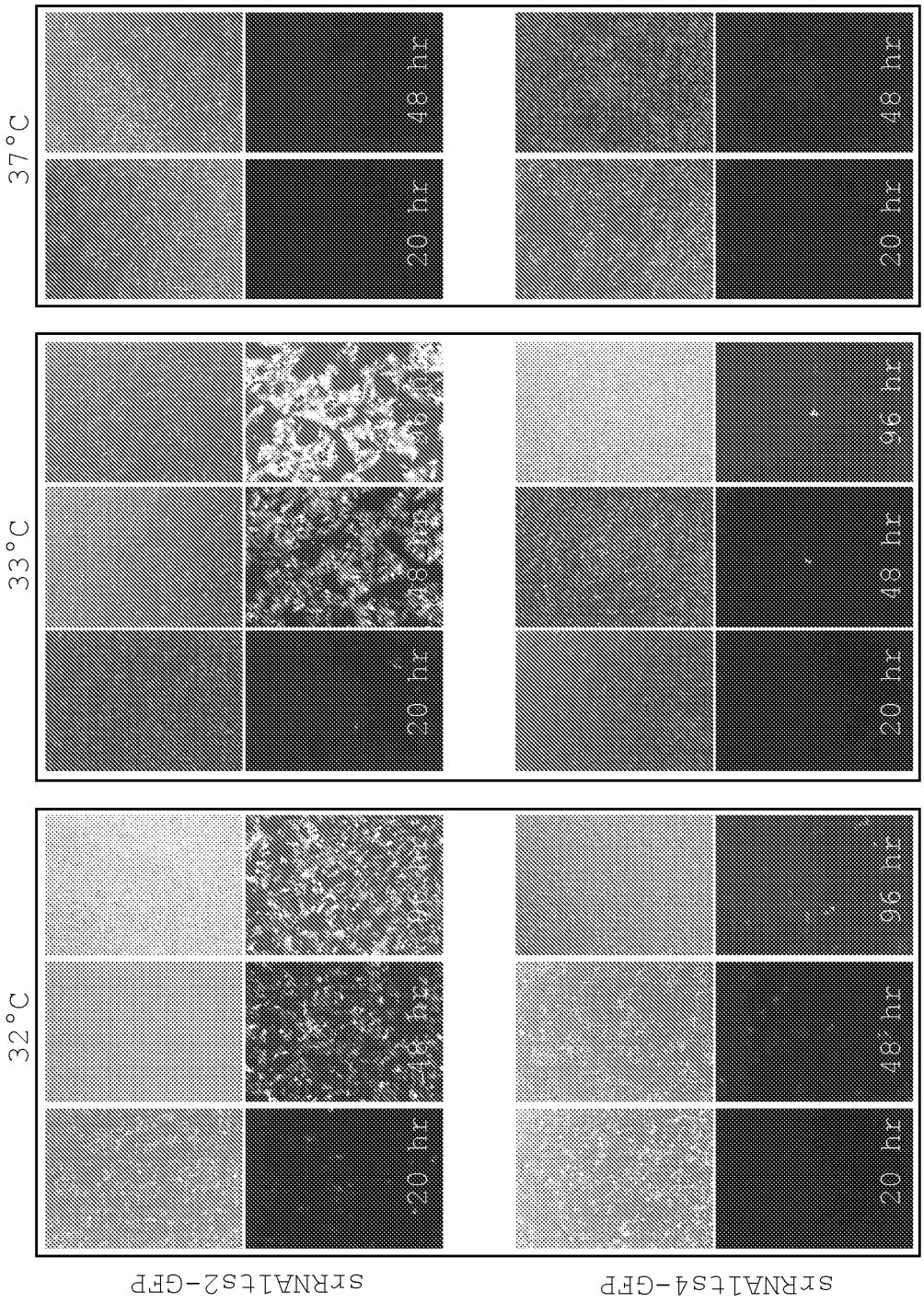


FIG. 6

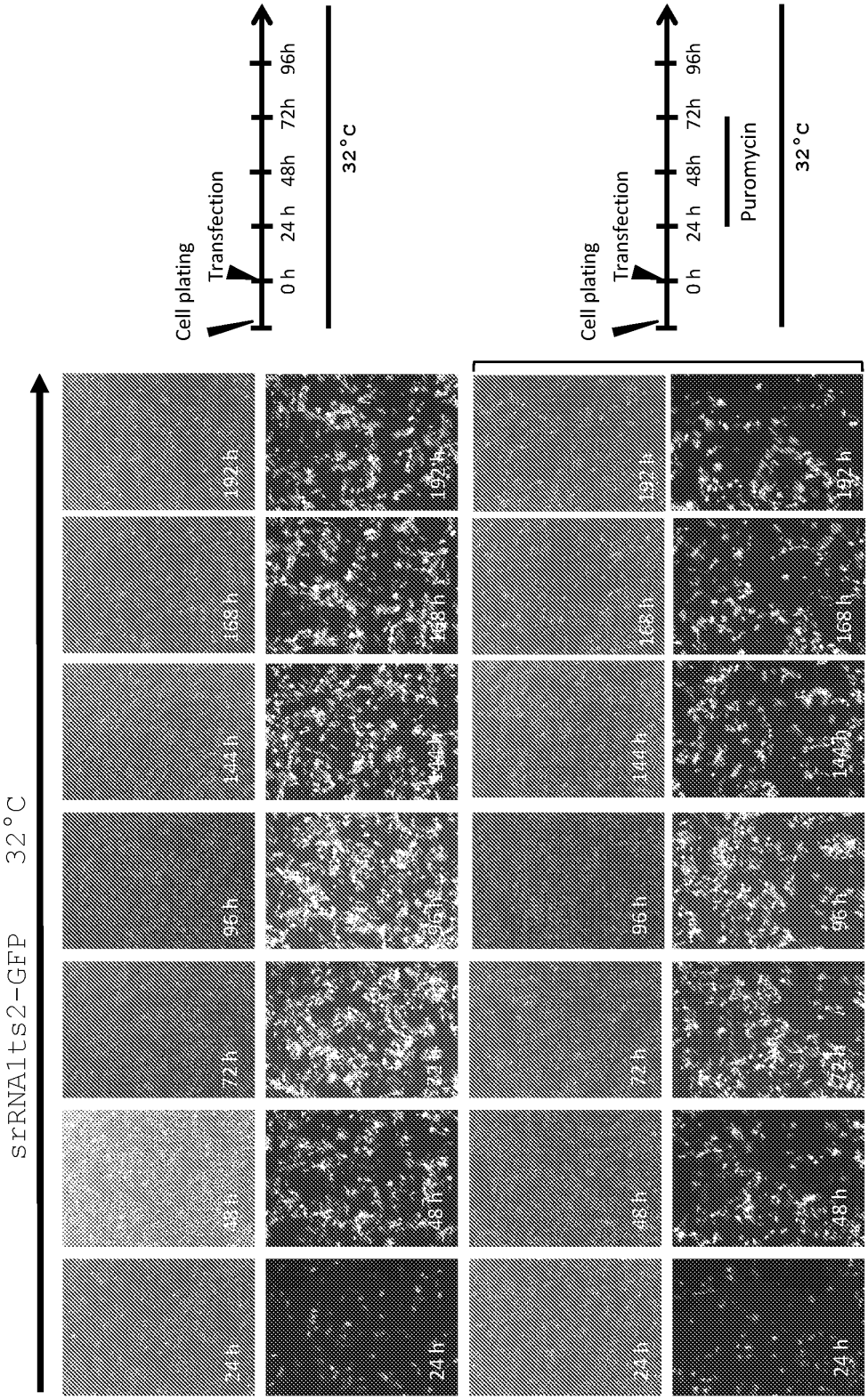

srRNA1ts2-GFP 32°C
Cell plating
Transfection
0 h
24 h
48h
72h
96h
32°c
Puromycin
24 h
48 h
72 h
96 h
144 h
168 h
192 h

FIG. 7

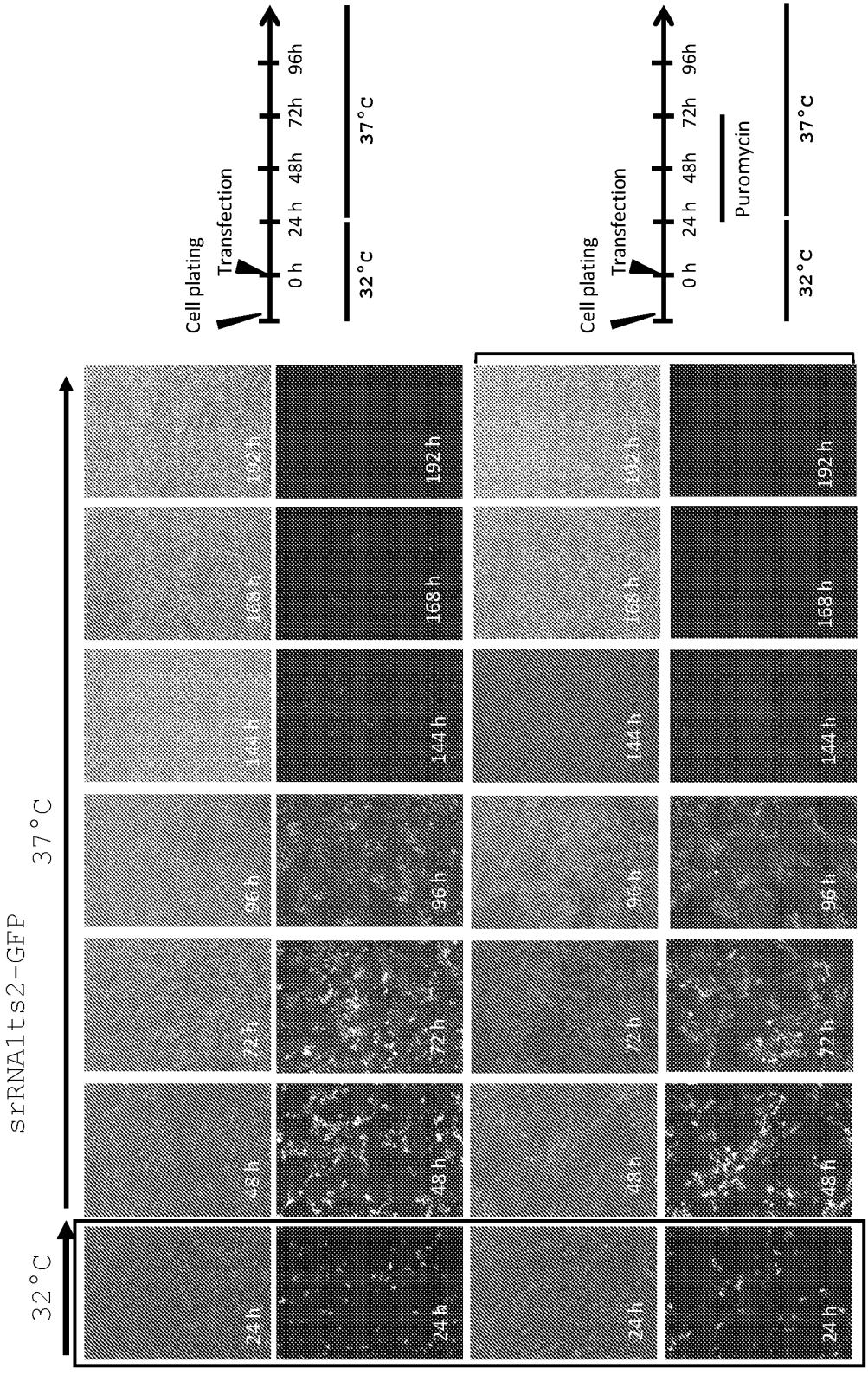

Cell plating
Transfection
0 h
24 h
48h
72h
96h
32°C
37°C
Puromycin
srRNAlts2-GFP
32°C
37°C
24 h
48 h
72 h
96 h
144 h
168 h
192 h

FIG. 8

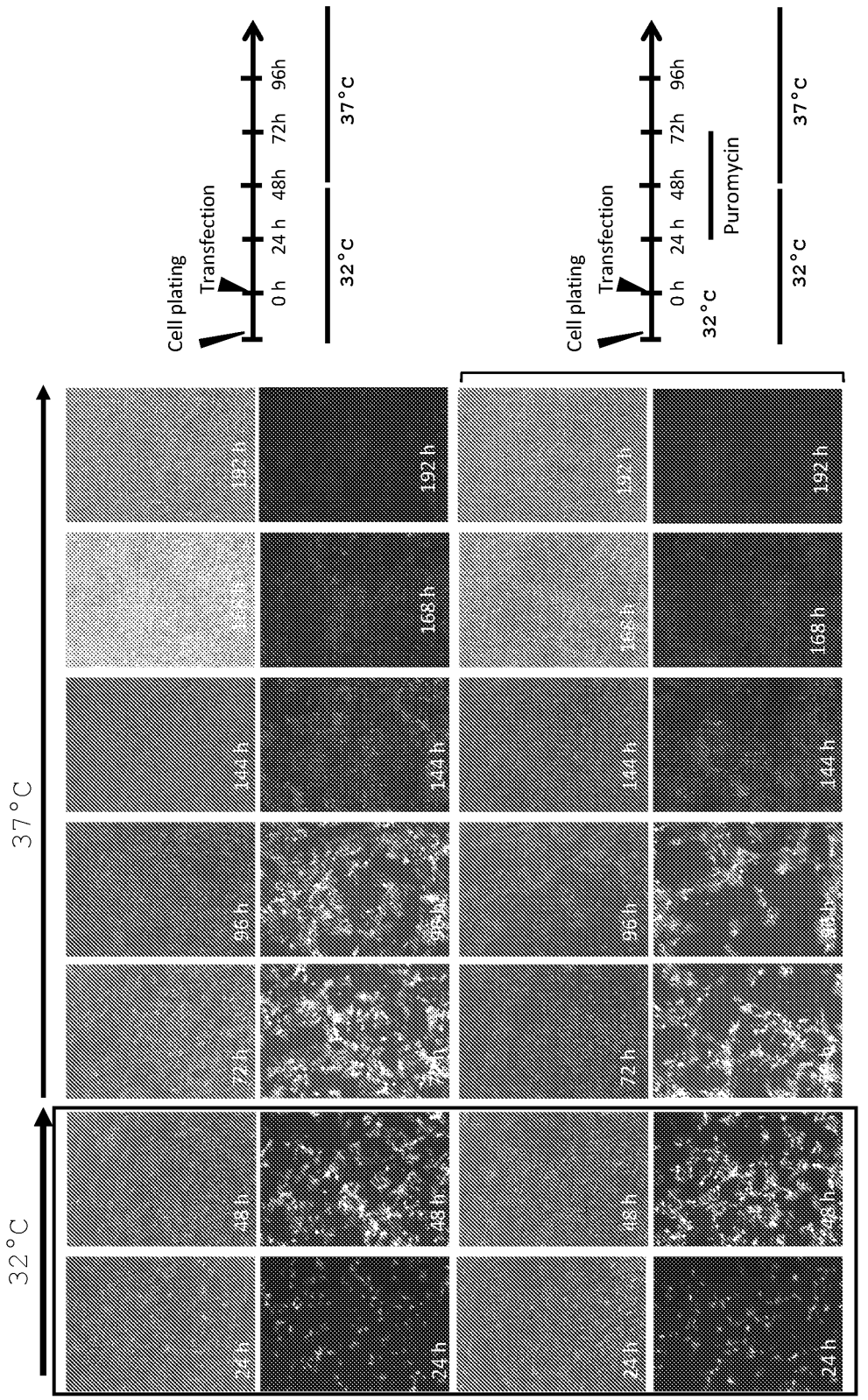

srRNA1ts2-GFP
32°C
37°C
24 h
48 h
72 h
96 h
120 h
144 h
168 h
192 h
Cell plating
Transfection
0 h
24 h
48h
72h
96h
32°c
37°c
Puromycin

FIG. 9

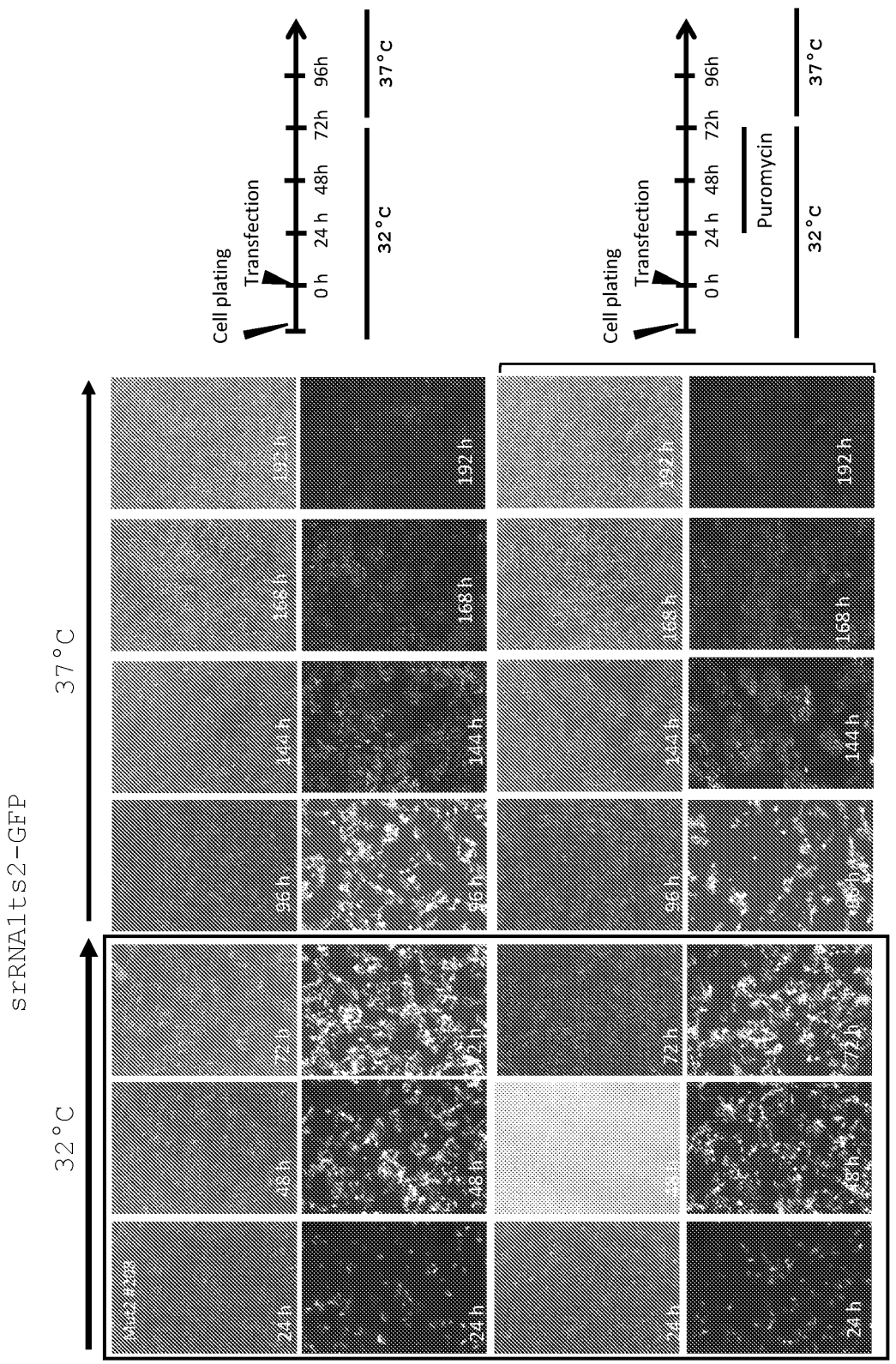

srRNA1ts2-GFP
32°C
37°C
24 h
48 h
72 h
96 h
144 h
168 h
192 h
Cell plating
Transfection
0 h
24 h
48h
72h
96h
32°C
37°C
Puromycin

FIG. 10

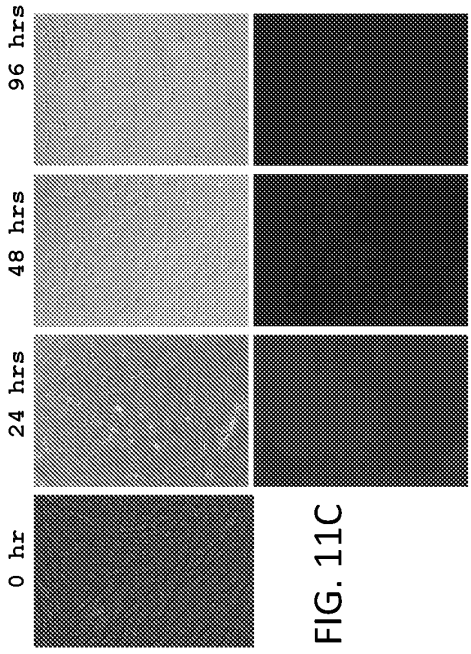


FIG. 11C

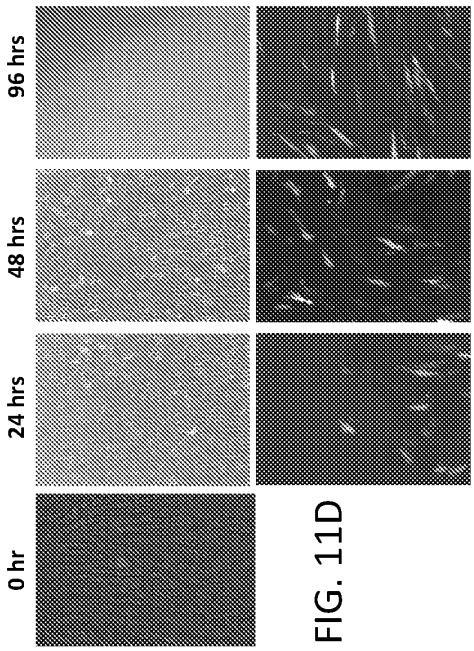


FIG. 11D

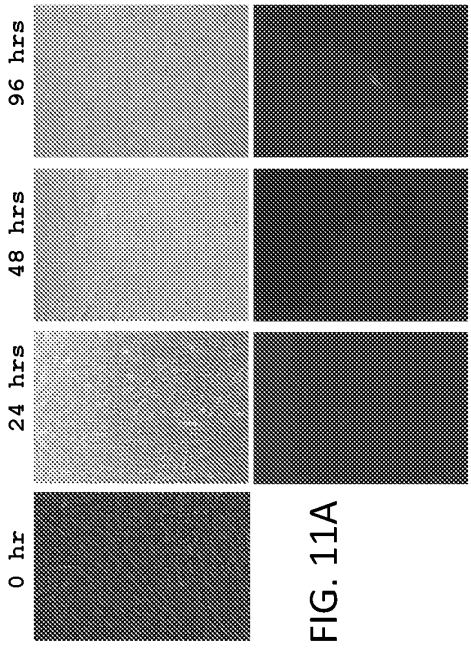


FIG. 11A

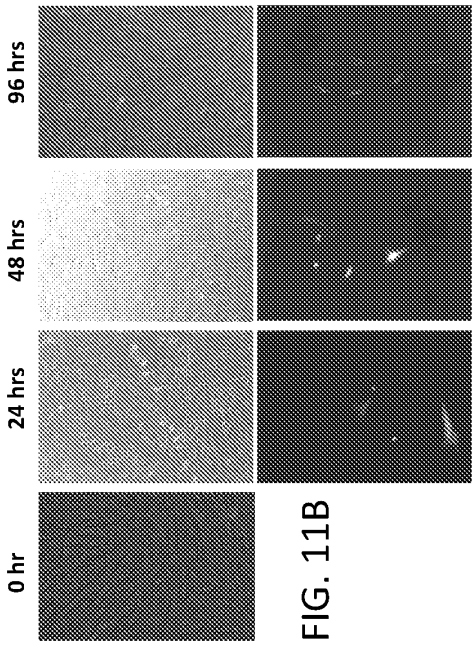


FIG. 11B

SEQ ID NO:
580 β5
β6 592
VEEV GRVYDMNTGTLRN 21
Aura GDQILPIYGRVSV 22
WEEV GRVADIRNNTIKD 23
BFV GMQIVVTEMRIQR 24
ONNV NKQICITTRKVDE 25
RRV GLQVNVPERKVQP 26
SFV GKQAVIAERKIQP 27
SINV GTQLDLQTGRTRV 28
* ** * **
SEQ ID NO:
GRVYDMNTGAAATGTLRN 29
GDQILPITGAAAYGRVSV 30
GRVADIRTGAAANNTIKD 31
GMQIVVTTGAAAEMRIQR 32
NKQICITTGAAATRKVDE 33
GLQVNVPTGAAAERKVQP 34
GKQAVIATGAAAERKIQP 35
GTQLDLQTGAAATGRTRV 36

FIG. 12

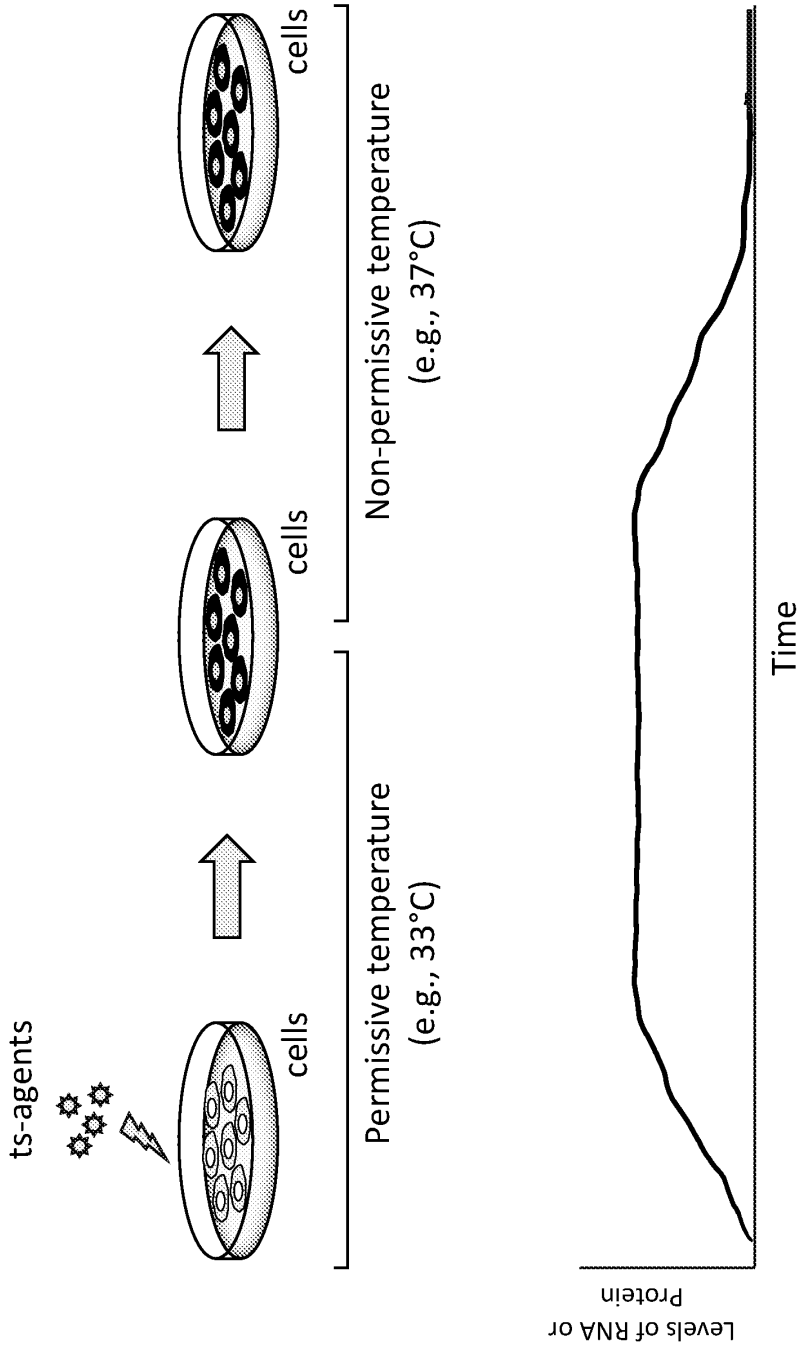

ts-agents
cells
Permissive temperature
(e.g., 33°C)
cells
Non-permissive temperature
(e.g., 37°C)
cells
Levels of RNA or
Protein
Time

FIG. 13

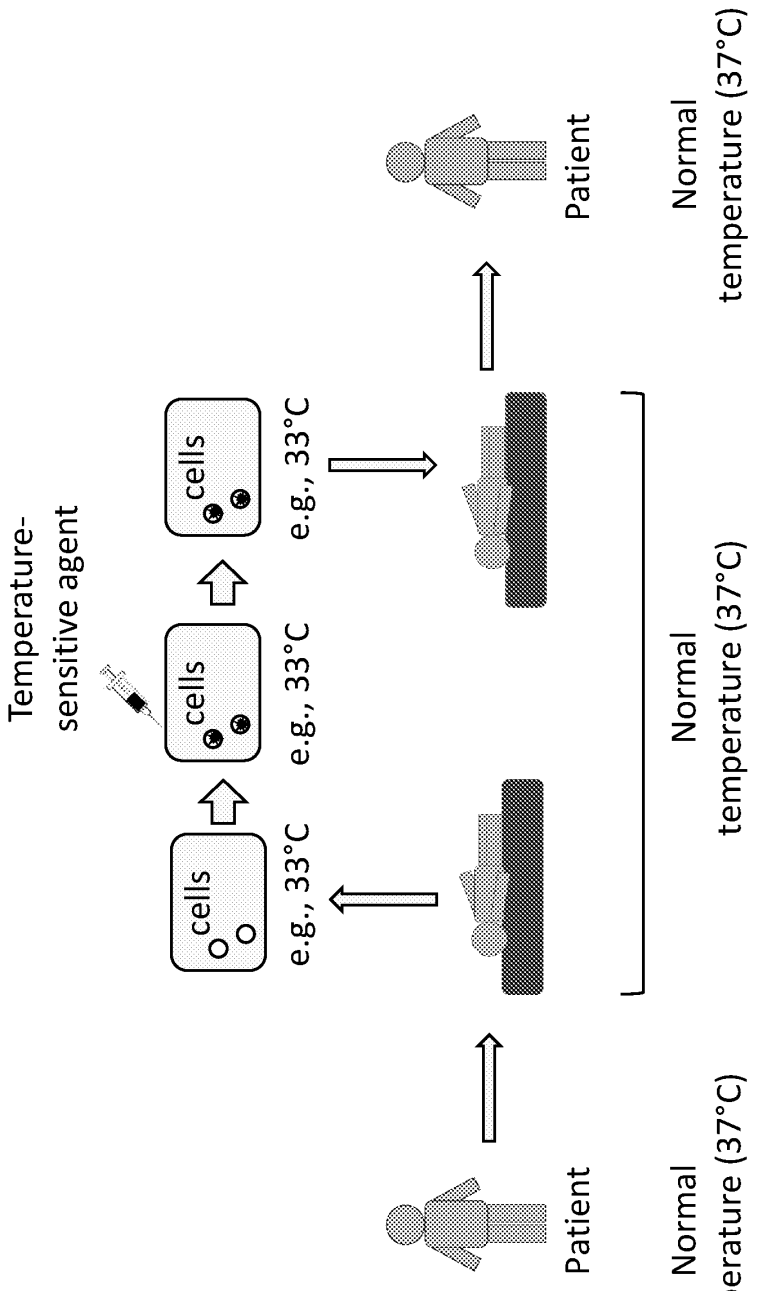

Patient
Normal temperature (37°C)
e.g., 33°C
cells
Temperature-sensitive agent
e.g., 33°C
cells
e.g., 33°C
cells
Normal temperature (37°C)
Patient
Normal temperature (37°C)

FIG. 14

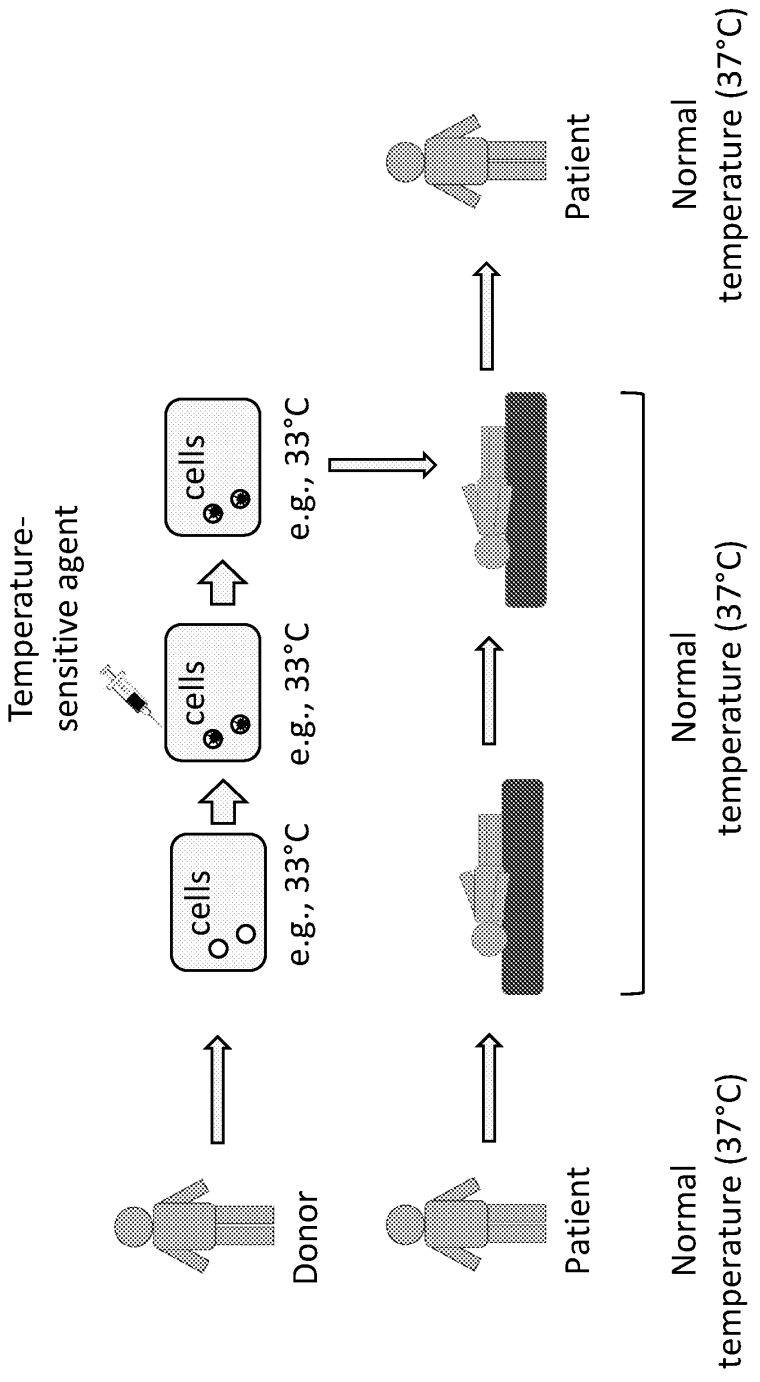

Temperature-
sensitive agent
cells
cells
cells
e.g., 33°C
e.g., 33°C
e.g., 33°C
Donor
Patient
Patient
Normal
temperature (37°C)
Normal
temperature (37°C)
Normal
temperature (37°C)

FIG. 15

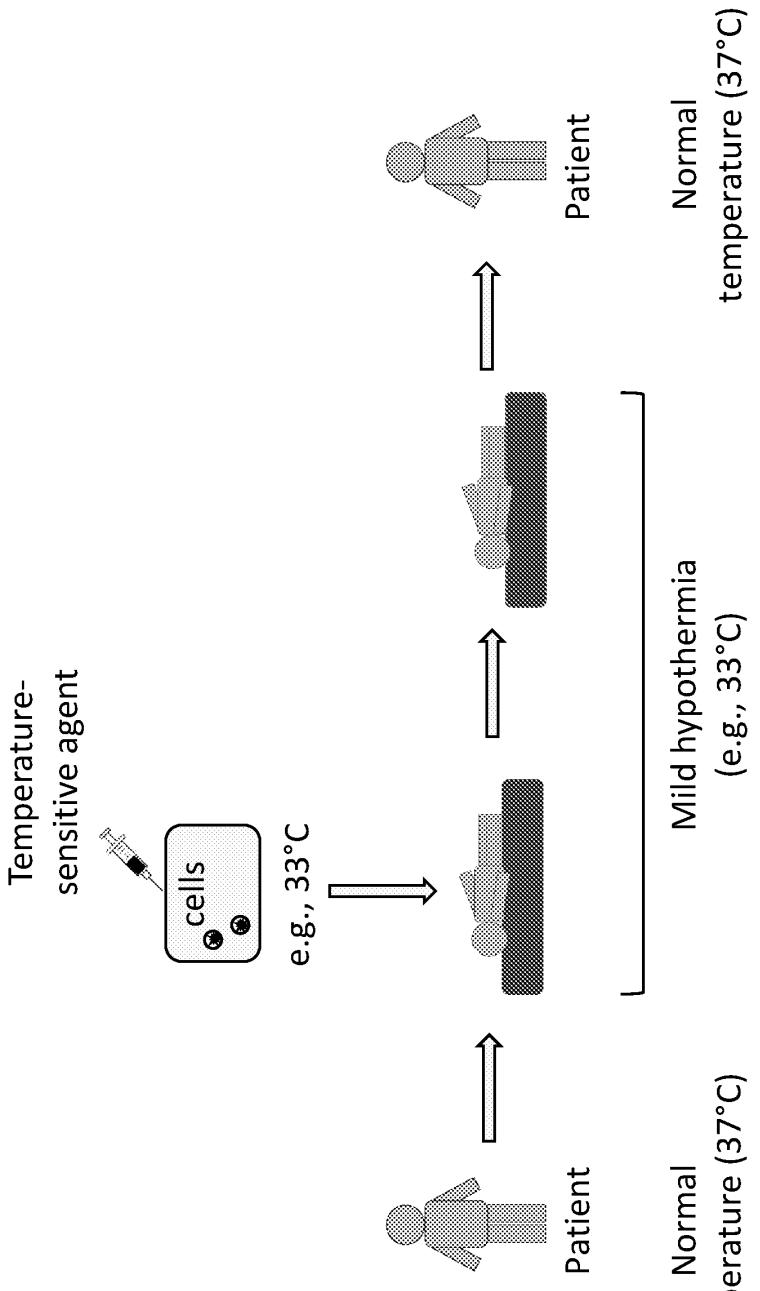

Temperature-sensitive agent
cells
e.g., 33°C
Patient
Normal temperature (37°C)
Mild hypothermia (e.g., 33°C)
Patient
Normal temperature (37°C)

FIG. 16

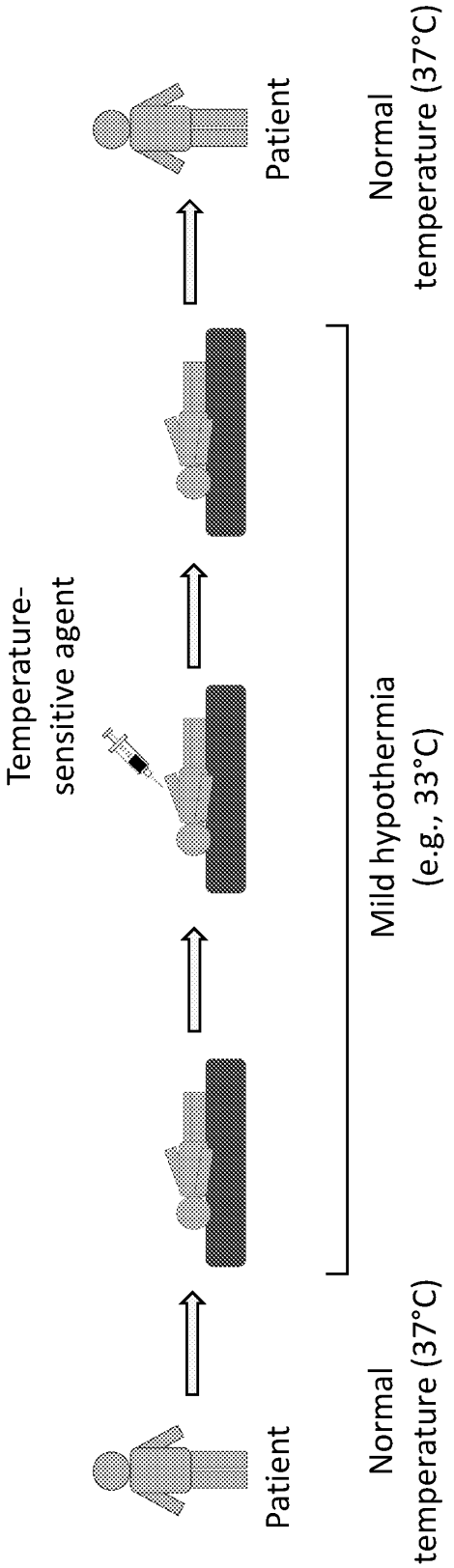

Patient
Normal temperature (37°C)
Temperature-sensitive agent
Mild hypothermia (e.g., 33°C)
Patient
Normal temperature (37°C)

FIG. 17

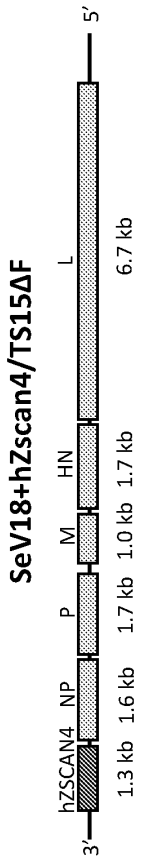

SeV18+hZscan4/TS15ΔF
3′
hZSCAN4
1.3 kb
NP
1.6 kb
P
1.7 kb
M
1.0 kb
HN
1.7 kb
L
6.7 kb
5′

FIG. 18

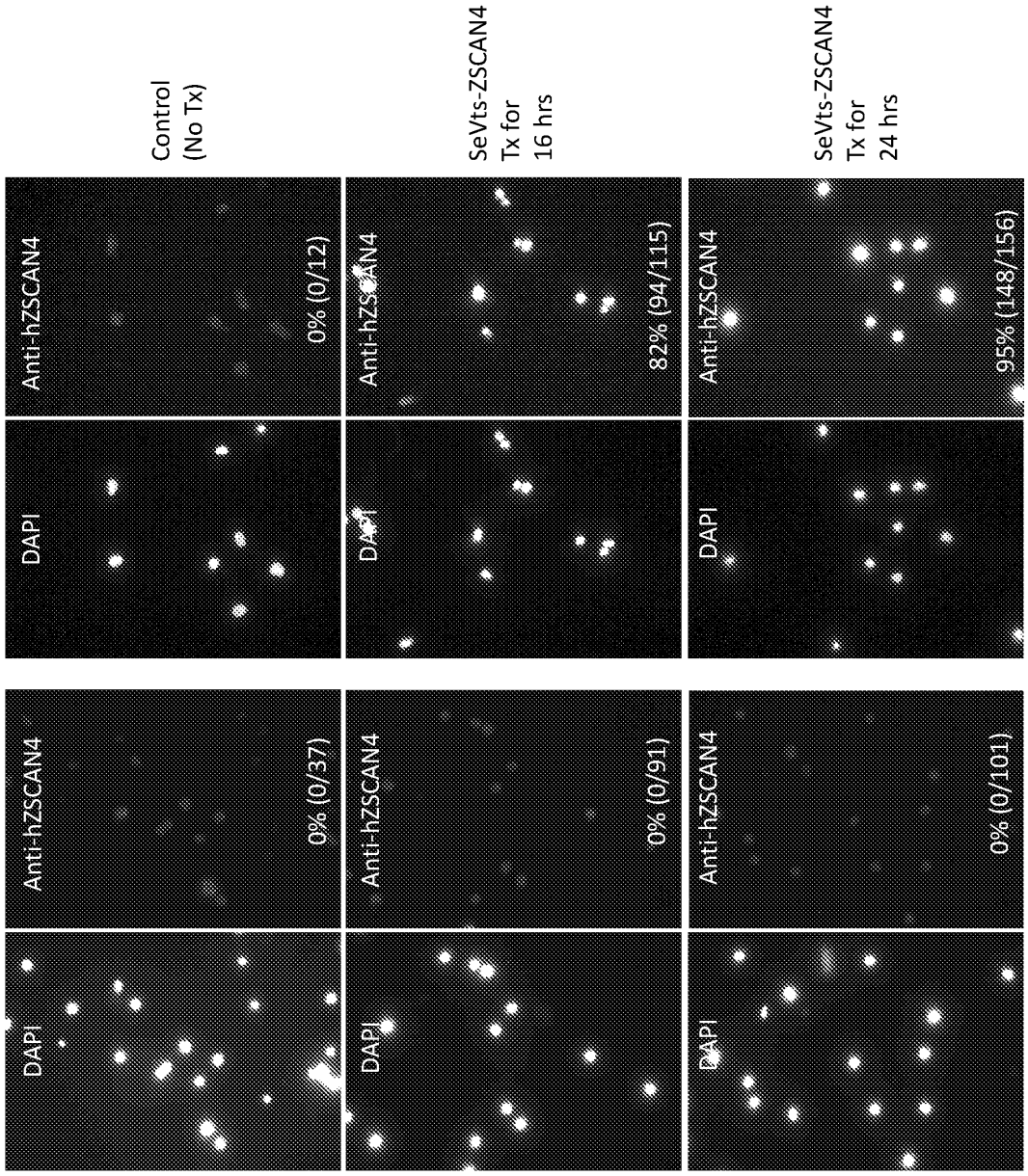

Control (No Tx)
SeVts-ZSCAN4 Tx for 3 hrs
SeVts-ZSCAN4 Tx for 6 hrs
Control (No Tx)
SeVts-ZSCAN4 Tx for 16 hrs
SeVts-ZSCAN4 Tx for 24 hrs
DAPI
Anti-hZSCAN4
0% (0/37)
0% (0/91)
0% (0/101)
0% (0/12)
82% (94/115)
95% (148/156)

FIG. 19

FIG. 20A

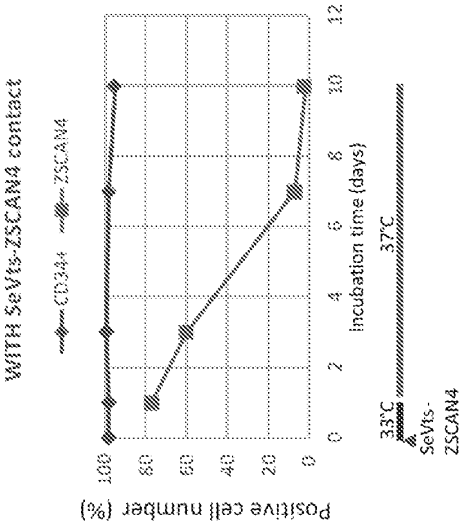
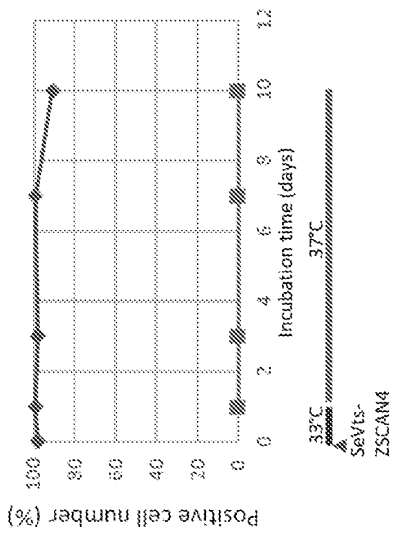


FIG. 20B



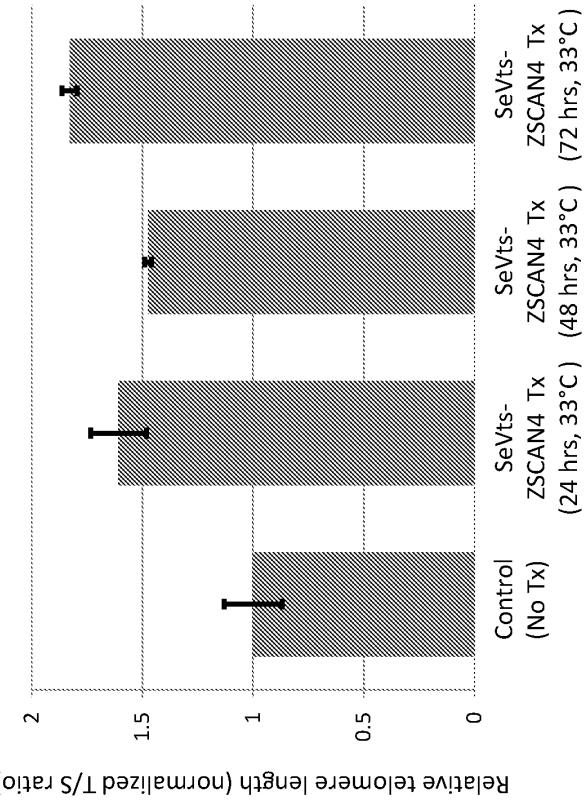

Relative telomere length (normalized T/S ratio)
2
1.5
1
0.5
0
Control (No Tx)
SeVts-ZSCAN4 Tx (24 hrs, 33°C)
SeVts-ZSCAN4 Tx (48 hrs, 33°C)
SeVts-ZSCAN4 Tx (72 hrs, 33°C)

FIG. 21

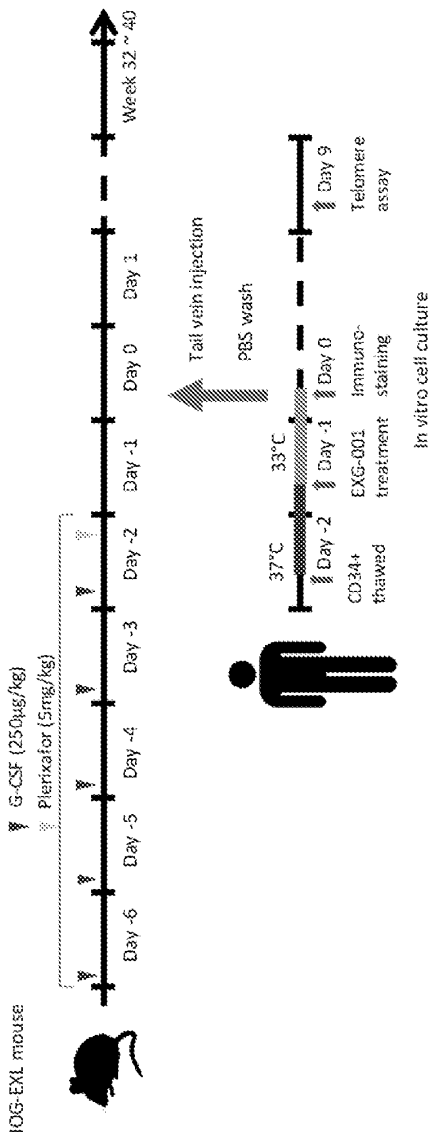

NOG-EXL mouse
G-CSF (250μg/kg)
Plerixafor (5mg/kg)
Day -6
Day -5
Day -4
Day -3
Day -2
Day -1
Day 0
Day 1
Week 32 ~ 40
Tail vein injection
PBS wash
37°C
33°C
Day -2
Day -1
Day 0
Day 9
CD34+ thawed
EXG-001 treatment
Immuno-staining
Telomere assay
In vitro cell culture

FIG. 22

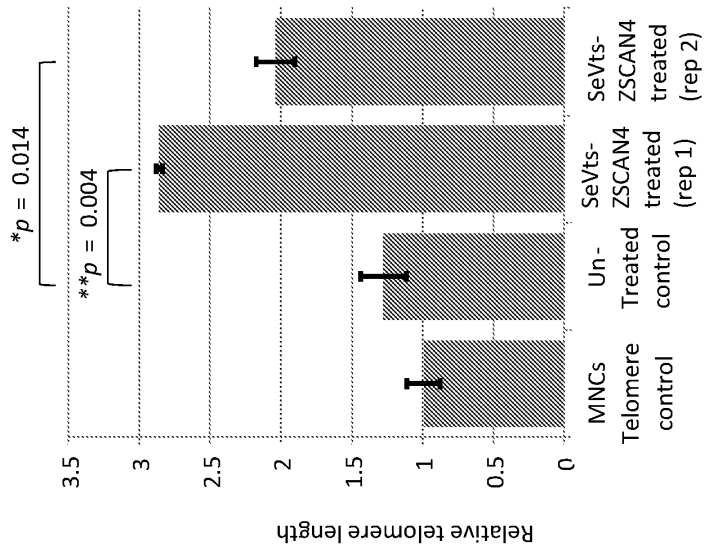

*p = 0.014
**p = 0.004
3.5
3
2.5
2
1.5
1
0.5
0
Relative telomere length
MNCs Telomere control
Un-Treated control
SeVts-ZSCAN4 treated (rep 1)
SeVts-ZSCAN4 treated (rep 2)

FIG. 23

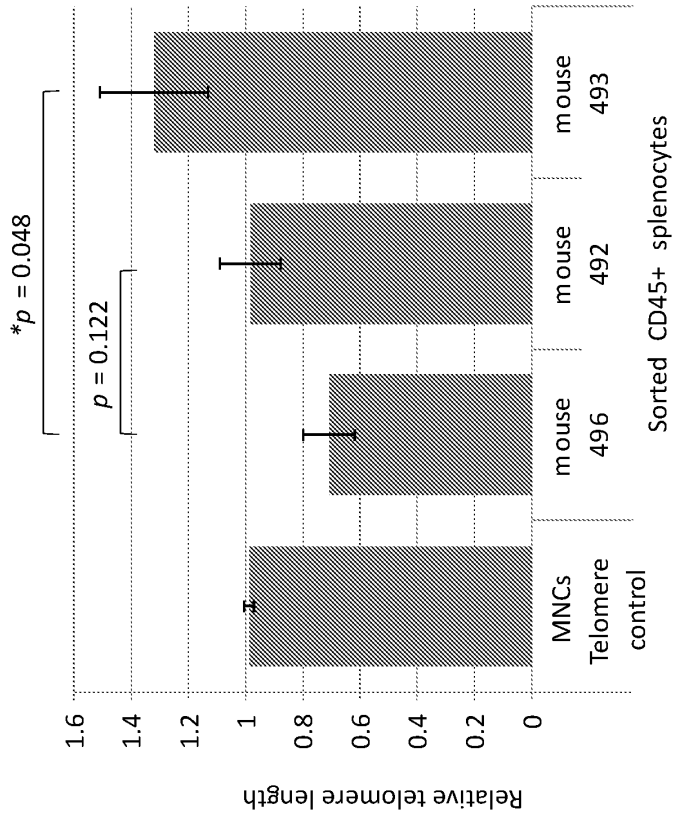

*p = 0.048
p = 0.122
Relative telomere length
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
MNCs
Telomere control
mouse 496
mouse 492
mouse 493
Sorted CD45+ splenocytes

FIG. 24

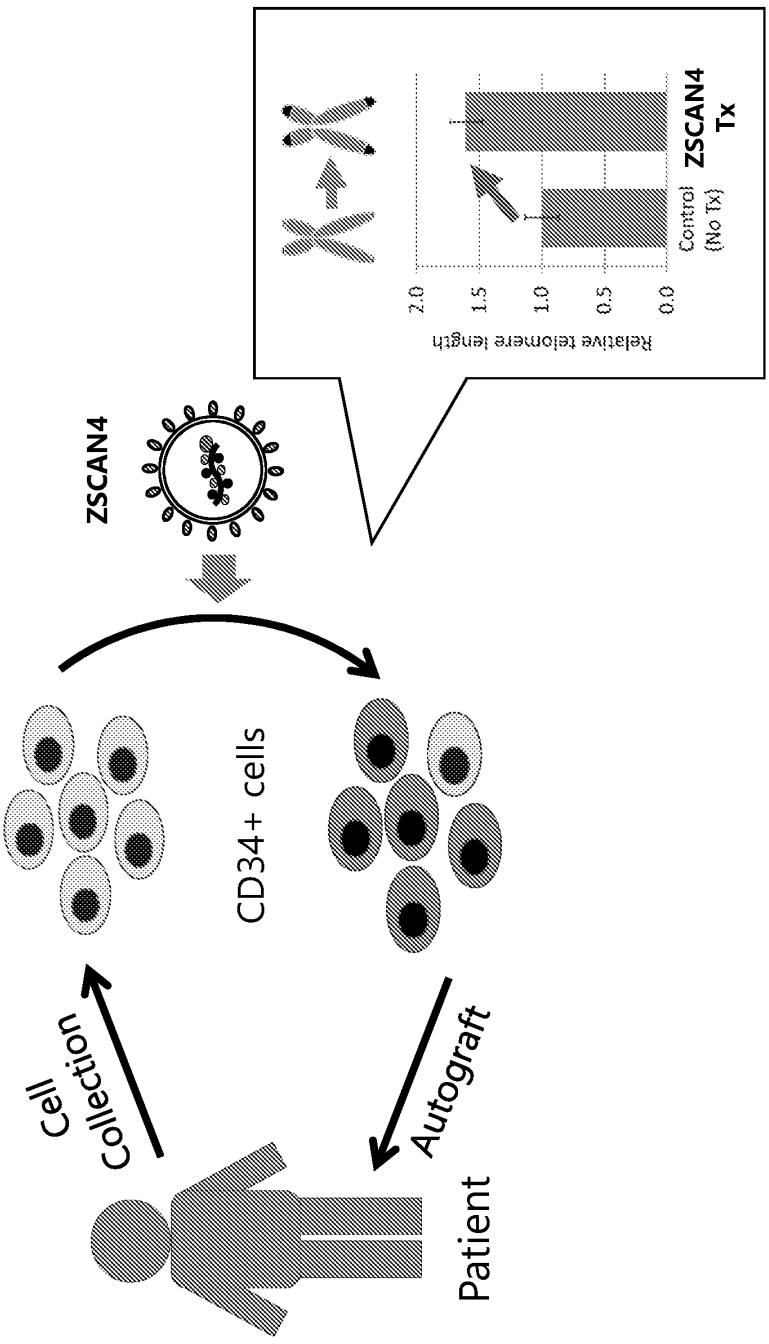

ZSCAN4
CD34+ cells
Cell Collection
Autograft
Patient
Relative telomere length
2.0
1.5
1.0
0.5
0.0
Control (No Tx)
ZSCAN4 Tx

FIG. 25

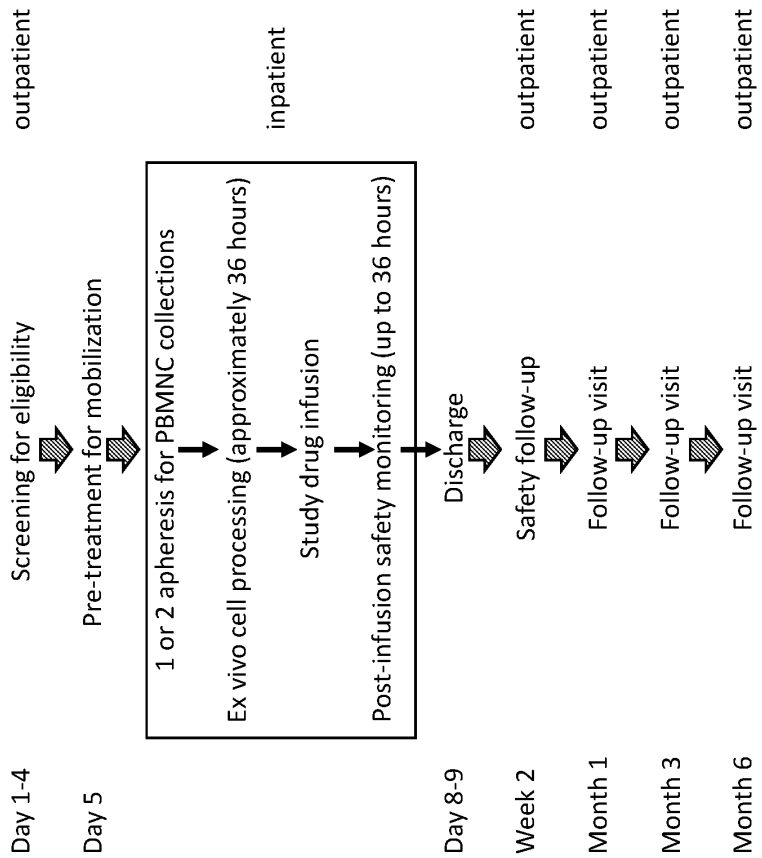

Day 1-4
Screening for eligibility
outpatient
Day 5
Pre-treatment for mobilization
1 or 2 apheresis for PBMNC collections
Ex vivo cell processing (approximately 36 hours)
Study drug infusion
Post-infusion safety monitoring (up to 36 hours)
inpatient
Day 8-9
Discharge
Week 2
Safety follow-up
outpatient
Month 1
Follow-up visit
outpatient
Month 3
Follow-up visit
outpatient
Month 6
Follow-up visit
outpatient

FIG. 26

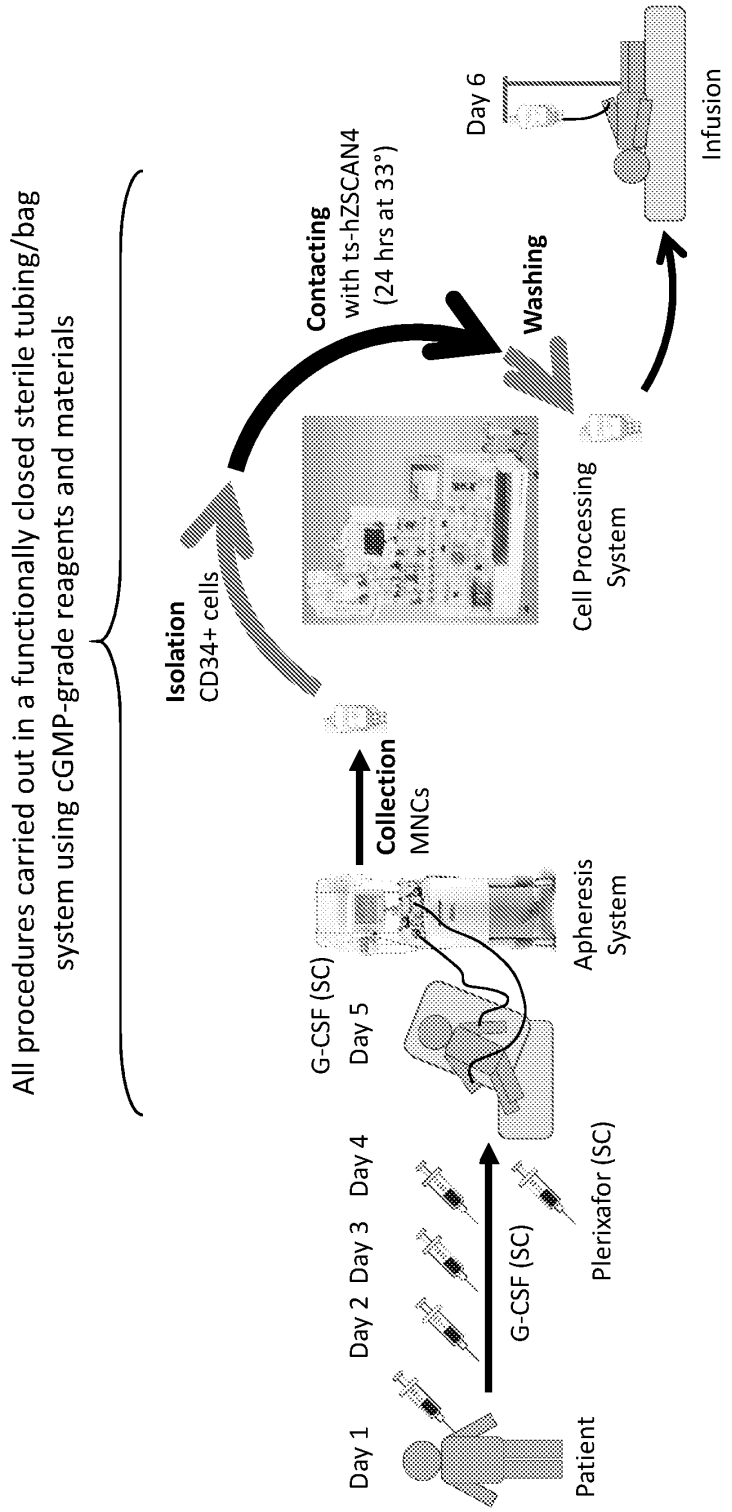

All procedures carried out in a functionally closed sterile tubing/bag system using cGMP-grade reagents and materials
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Patient
G-CSF (SC)
G-CSF (SC)
Plerixafor (SC)
Apheresis System
Collection MNCs
Isolation CD34+ cells
Contacting with ts-hZSCAN4 (24 hrs at 33°)
Washing
Cell Processing System
Infusion

FIG. 27

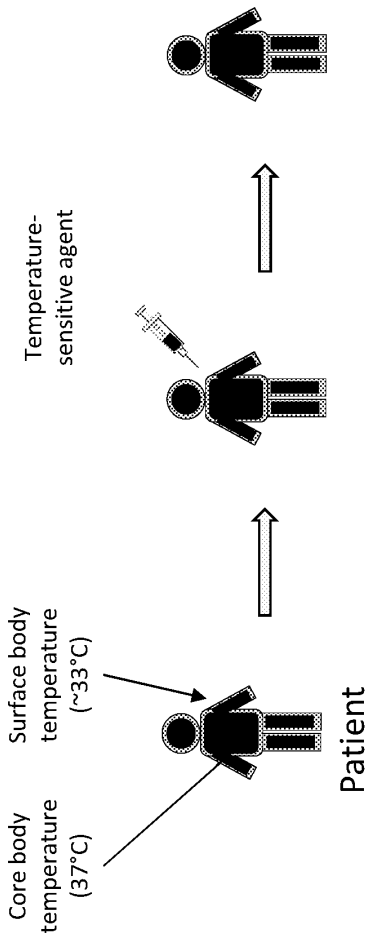

Core body temperature (37°C)
Surface body temperature (~33°C)
Patient
Temperature-sensitive agent

FIG. 28

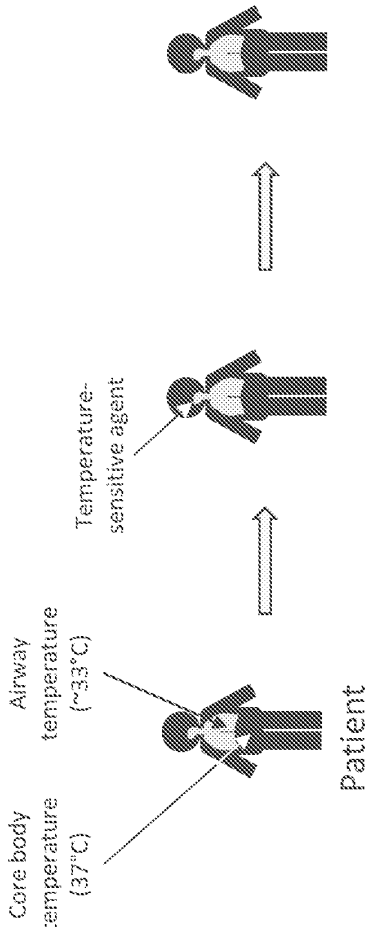

Core body temperature (37°C)
Airway temperature (~33°C)
Patient
Temperature-sensitive agent

FIG. 29