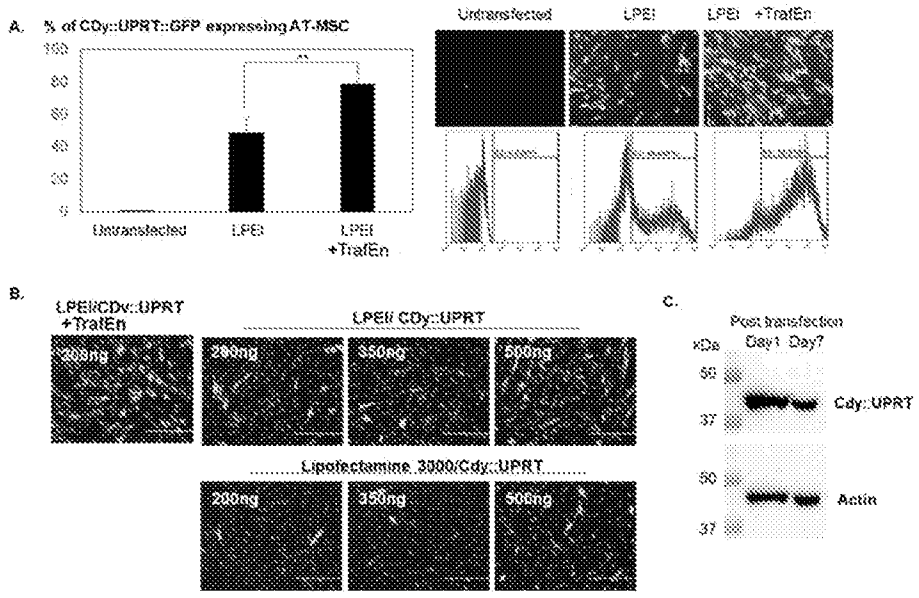

A.
% of CDy::UPRT::GFP expressing AT-MSC
Untransfected
LPEI
LPEI +TrafEn
B.
LPEI/CDy::UPRT +TrafEn
LPEI/ CDy::UPRT
200ng
350ng
500ng
Lipofectamine 3000/Cdy::UPRT
C.
Post transfection
Day1 Day7
kDa
Cdy::UPRT
Actin

FIG. 1

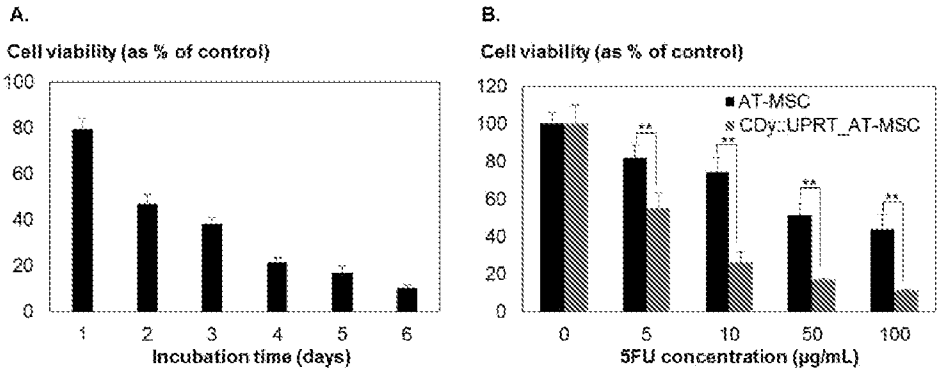

A.
Cell viability (as % of control)
Incubation time (days)
B.
Cell viability (as % of control)
AT-MSC
CDy::UPRT_AT-MSC
5FU concentration (µg/mL)

FIG. 2

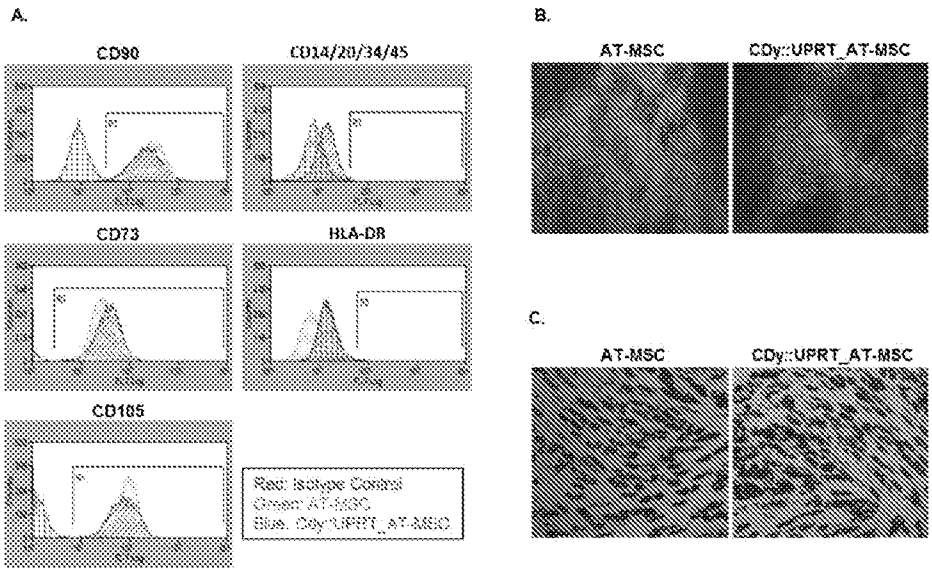

A.
CD90
CD14/20/34/45
CD73
HLA-DR
CD105
Red: Isotype Control
Green: AT-MSC
Blue: CDy::UPRT_AT-MSC
B.
AT-MSC
CDy::UPRT_AT-MSC
C.
AT-MSC
CDy::UPRT_AT-MSC

FIG. 3

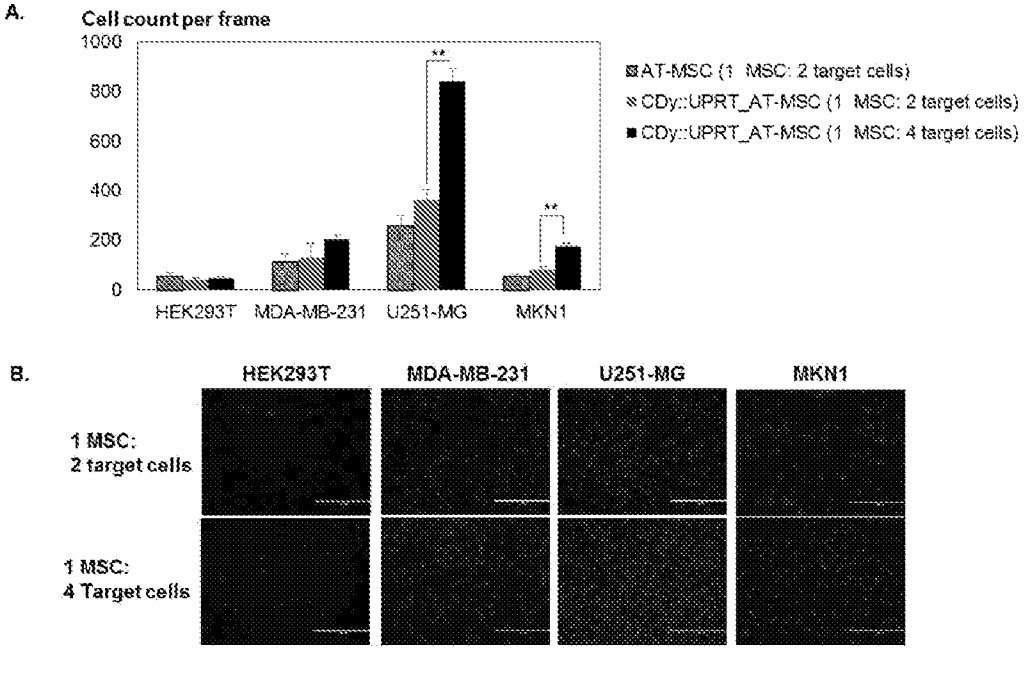

A.
Cell count per frame
1000
800
600
400
200
0
HEK293T
MDA-MB-231
U251-MG
MKN1
AT-MSC (1 MSC: 2 target cells)
CDy::UPRT_AT-MSC (1 MSC: 2 target cells)
CDy::UPRT_AT-MSC (1 MSC: 4 target cells)
B.
HEK293T
MDA-MB-231
U251-MG
MKN1
1 MSC:
2 target cells
1 MSC:
4 Target cells

FIG. 4

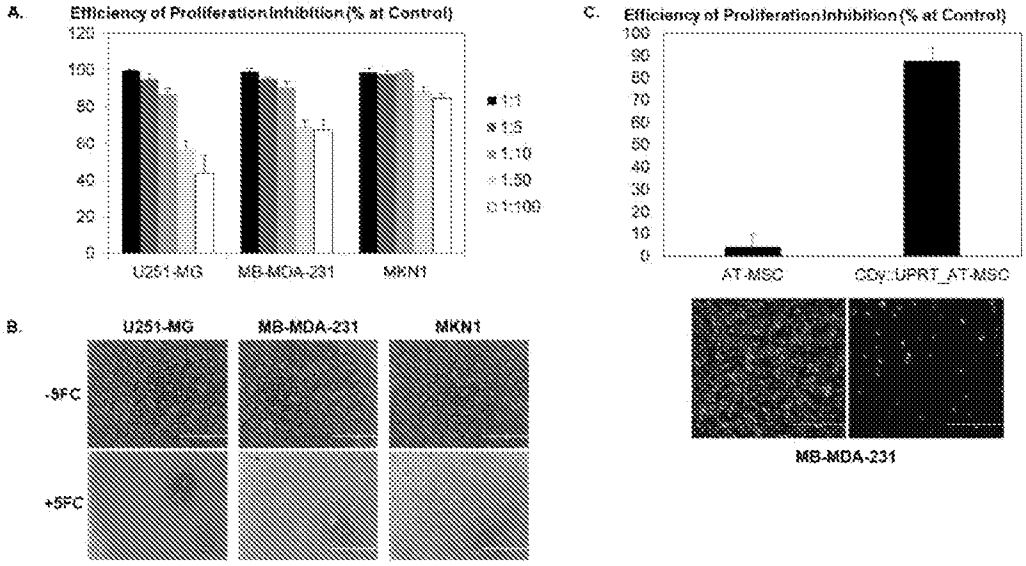

A.
Efficiency of Proliferation Inhibition (% at Control)
120
100
80
60
40
20
0
U251-MG
MB-MDA-231
MKN1
1:1
1:5
1:10
1:50
1:100
B.
U251-MG
MB-MDA-231
MKN1
-5FC
+5FC
C.
Efficiency of Proliferation Inhibition (% at Control)
100
90
80
70
60
50
40
30
20
10
0
AT-MSC
CDy::UPRT_AT-MSC
MB-MDA-231

FIG. 5

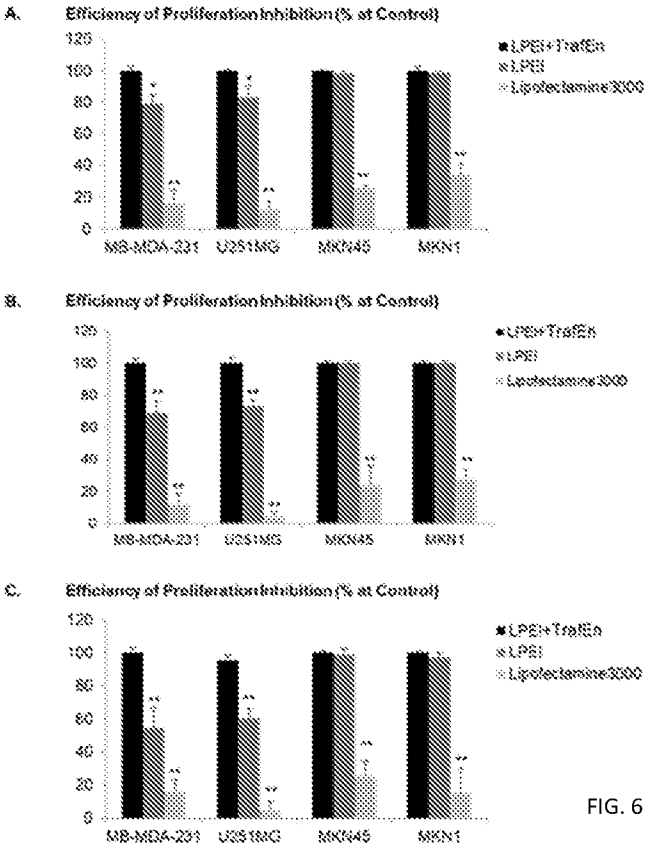

A.
Efficiency of Proliferation Inhibition (% at Control)
120
100
80
60
40
20
0
MB-MDA-231
U251MG
MKN45
MKN1
LPEI+TrailEn
LPEI
Lipofectamine3000
B.
Efficiency of Proliferation Inhibition (% at Control)
MB-MDA-231
U251MG
MKN45
MKN1
LPEI+TrailEn
LPEI
Lipofectamine3000
C.
Efficiency of Proliferation Inhibition (% at Control)
MB-MDA-231
U251MG
MKN45
MKN1
LPEI+TrailEn
LPEI
Lipofectamine3000

FIG. 6

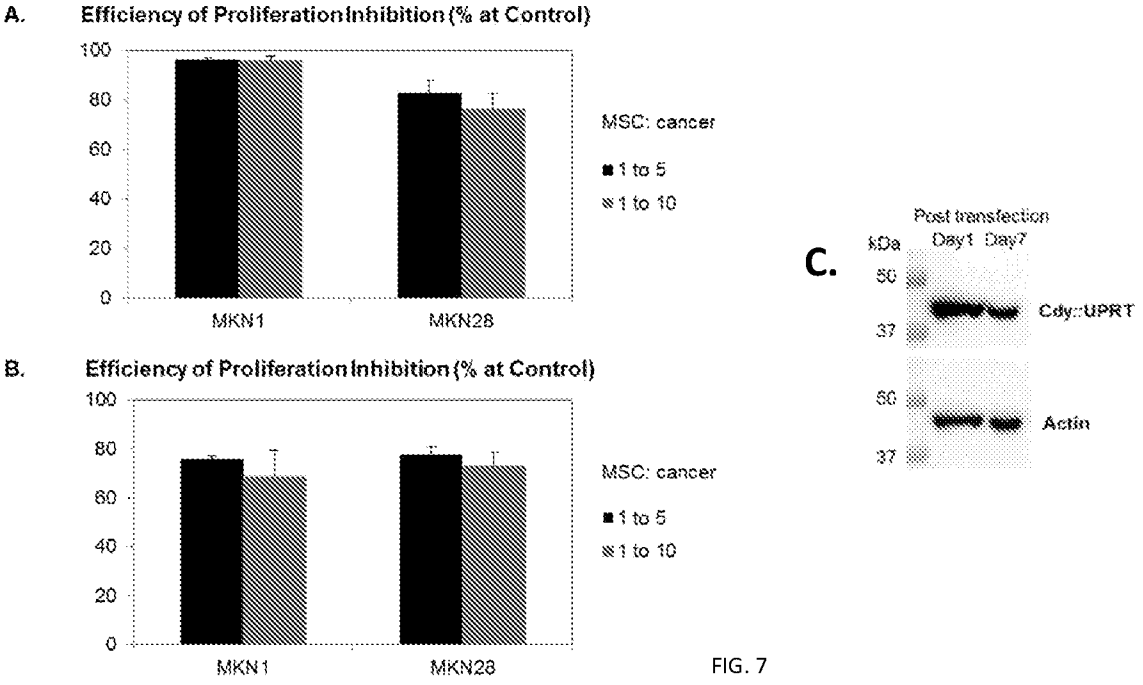

A.
Efficiency of Proliferation Inhibition (% at Control)
100
80
60
40
20
0
MKN1
MKN28
MSC: cancer
1 to 5
1 to 10
B.
Efficiency of Proliferation Inhibition (% at Control)
100
80
60
40
20
0
MKN1
MKN28
MSC: cancer
1 to 5
1 to 10
C.
Post transfection
kDa
Day1 Day7
50
37
Cdy::UPRT
50
37
Actin

FIG. 7

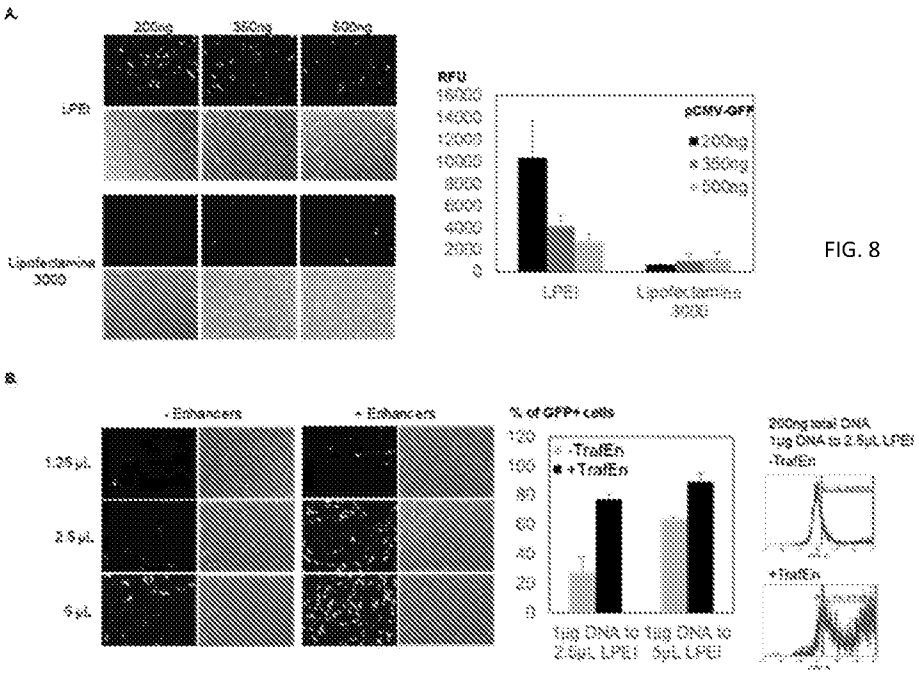

A.
LPEI
Lipofectamine 3000
RFU
pCMV-GFP
LPEI
Lipofectamine 3000
B.
- Enhancers
+ Enhancers
1.25 µL
2.5 µL
5 µL
% of GFP+ cells
- TrafEn
+ TrafEn
1µg DNA to 2.5µL LPEI
1µg DNA to 5µL LPEI
250ng total DNA
1µg DNA to 2.5µL LPEI
- TrafEn
+ TrafEn

FIG. 8

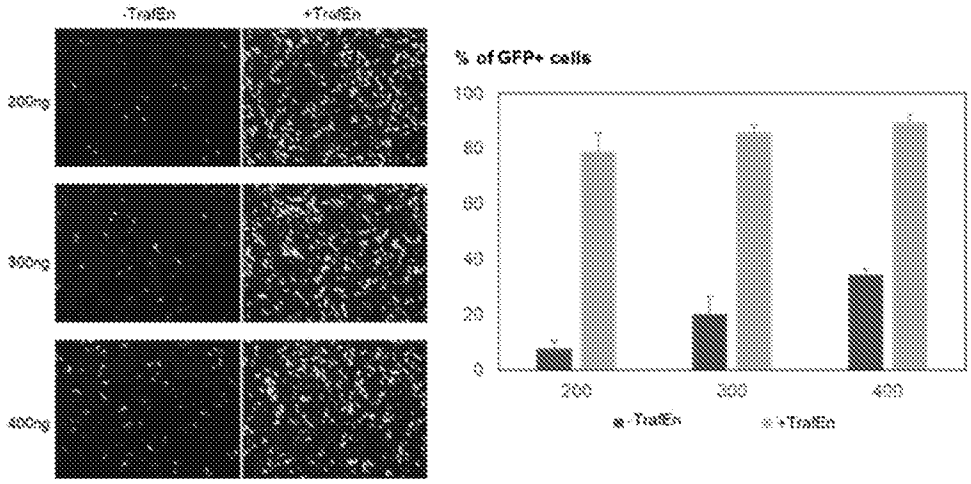


FIG. 9

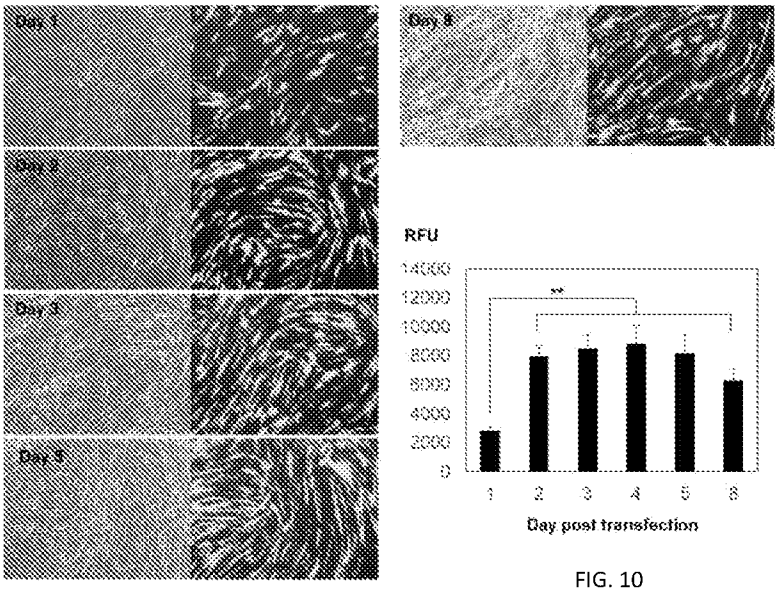


FIG. 10

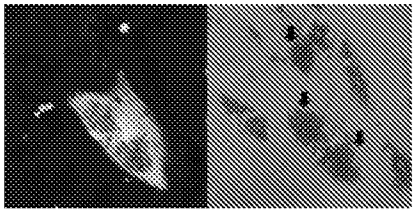


FIG. 11

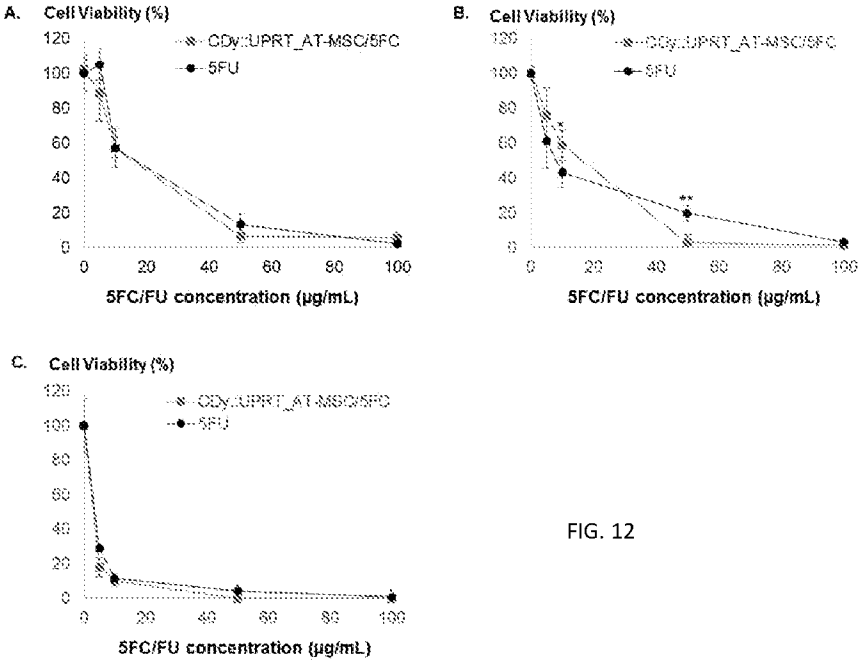

A.
Cell Viability (%)
CDy::UPRT_AT-MSC/5FC
5FU
5FC/FU concentration (μg/mL)
B.
Cell Viability (%)
CDy::UPRT_AT-MSC/5FC
5FU
5FC/FU concentration (μg/mL)
C.
Cell Viability (%)
CDy::UPRT_AT-MSC/5FC
5FU
5FC/FU concentration (μg/mL)

FIG. 12

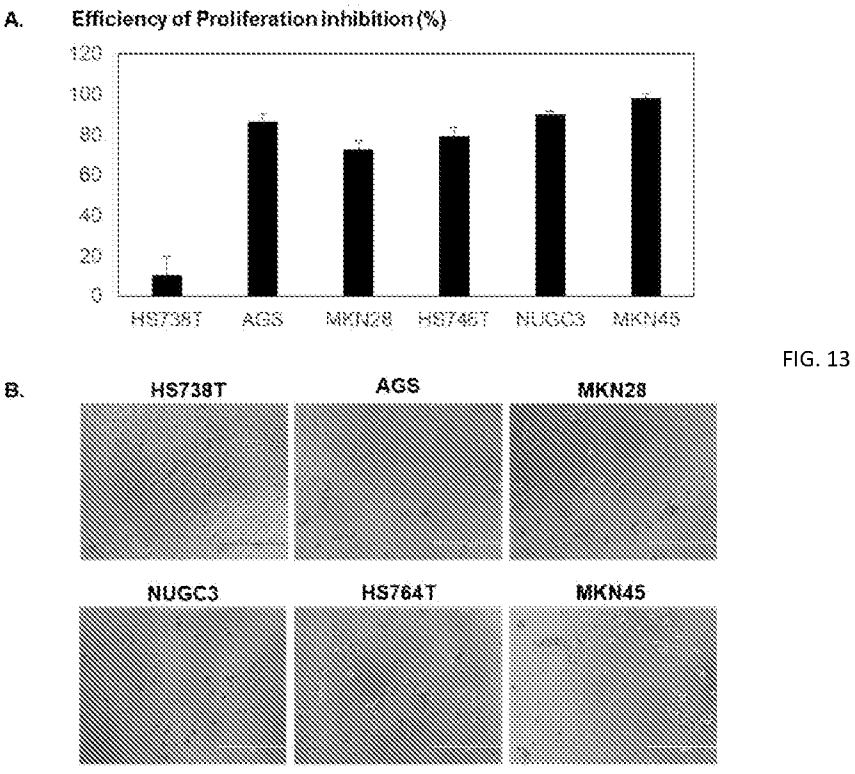

A.
Efficiency of Proliferation Inhibition (%)
HS738T
AGS
MKN28
HS746T
NUGC3
MKN45
B.
HS738T
AGS
MKN28
NUGC3
HS764T
MKN45

FIG. 13

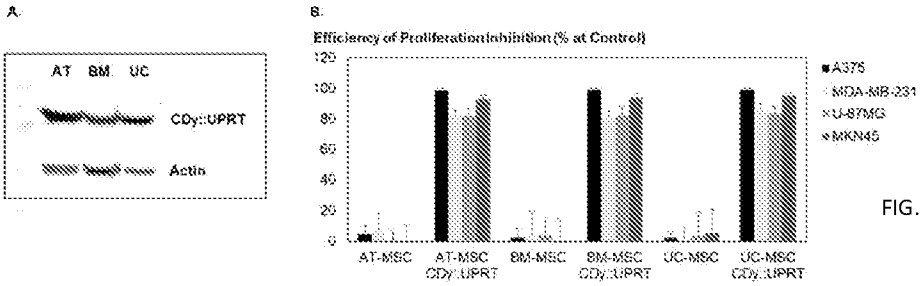


FIG. 14

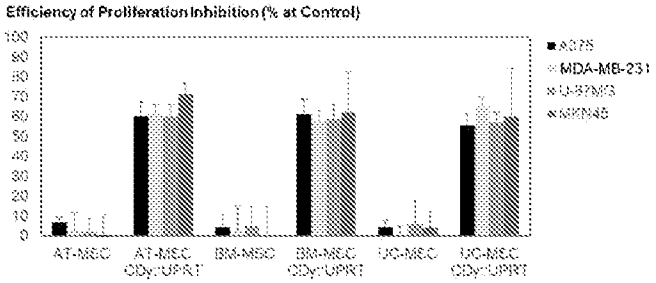


FIG. 15

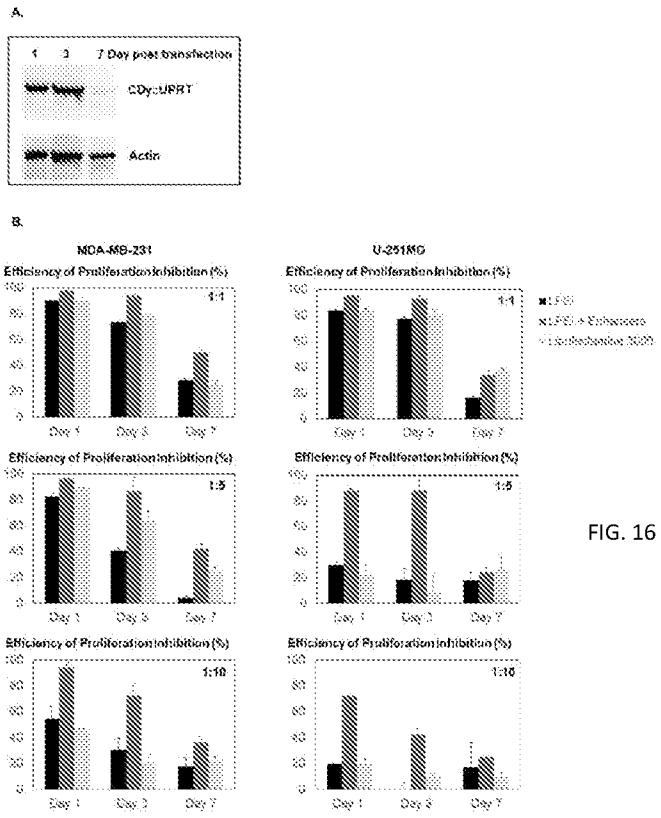


FIG. 16

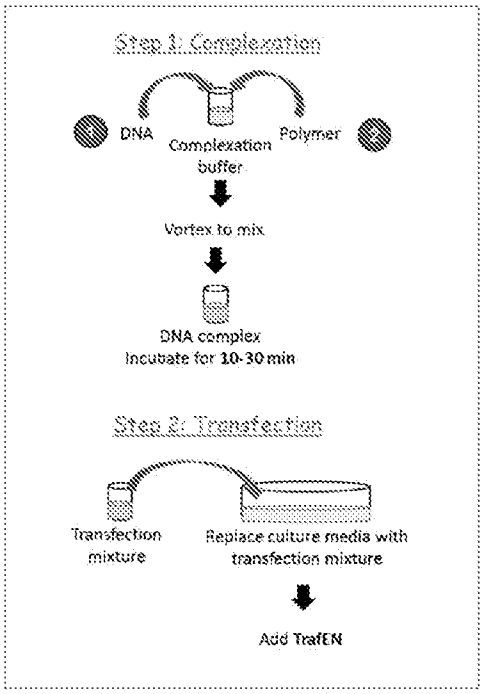

Step 1: Complexation
DNA
Complexation buffer
Polymer
Vortex to mix
DNA complex
Incubate for 10-30 min
Step 2: Transfection
Transfection mixture
Replace culture media with transfection mixture
Add TrafEN

FIG. 17

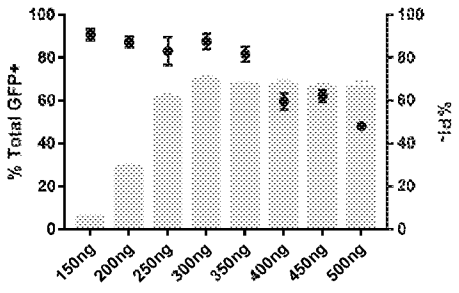
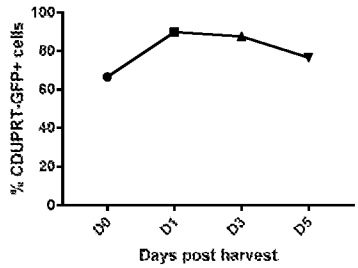

% Total GFP+
100
80
60
40
20
0
150ng
200ng
250ng
300ng
350ng
400ng
450ng
500ng

FIG. 18

FIG. 19


% CDUPRT-GFP+ cells
100
80
60
40
20
0
D0
D1
D3
D5
Days post harvest

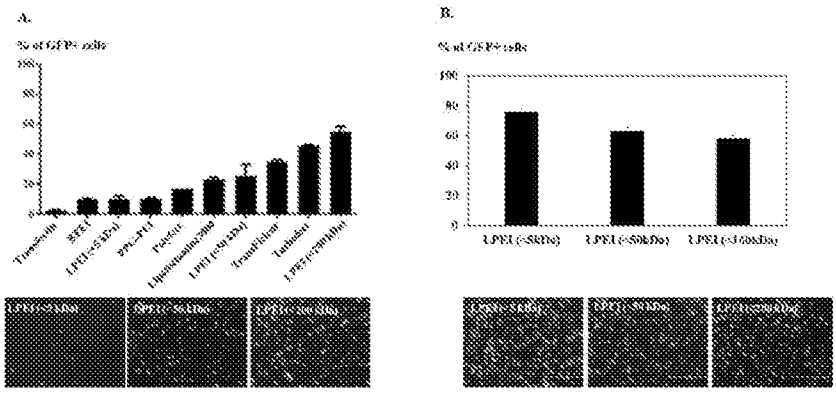

A.
B.

FIG. 20

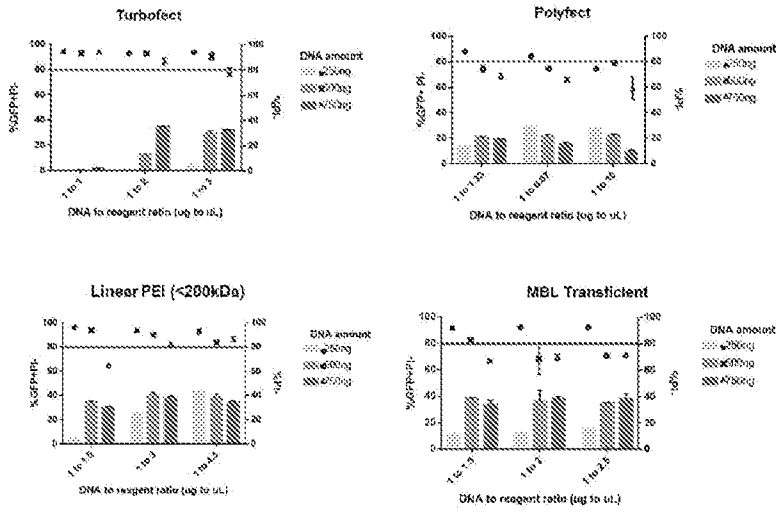
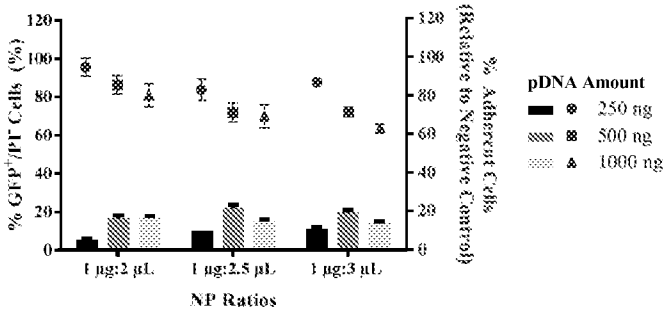

Turbofect
Polyfect
Linear PEI (<200kDa)
MBL Transficient
DNA amount
DNA to reagent ratio (ug to uL)

FIG. 21

FIG. 22


% GFP+/PI Cells (%)
% Adherent Cells (Relative to Negative Control)
pDNA Amount
250 ng
500 ng
1000 ng
1 µg:2 µL
1 µg:2.5 µL
1 µg:3 µL
NP Ratios

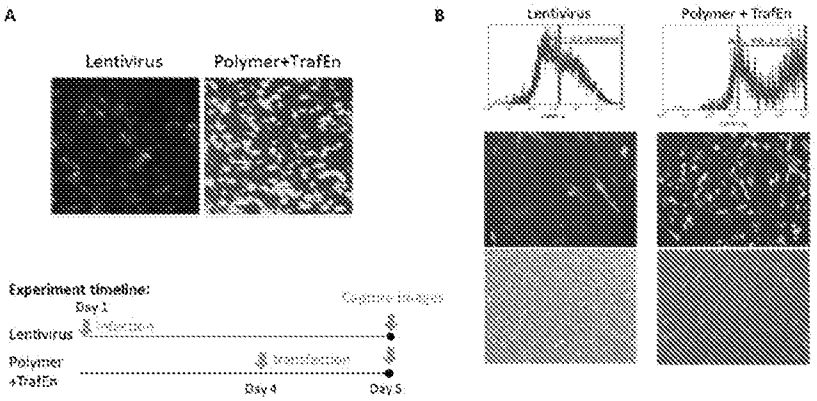

A
Lentivirus
Polymer+TrafEn
Experiment timeline:
Day 1
Lentivirus
Polymer +TrafEn
Day 4
Day 5
B
Lentivirus
Polymer + TrafEn

FIG. 23

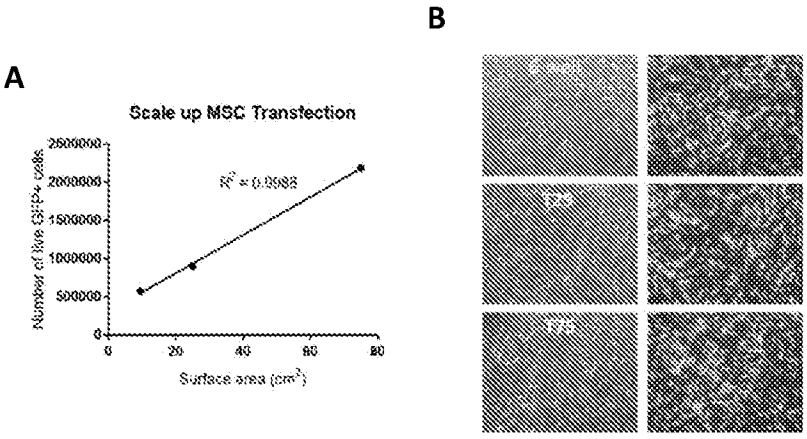

A
Scale up MSC Transfection
Number of live GFP+ cells
Surface area (cm²)
0
20
40
60
80
500000
1000000
1500000
2000000
2500000
B
Control
TrafEn
TrafEn

FIG. 24

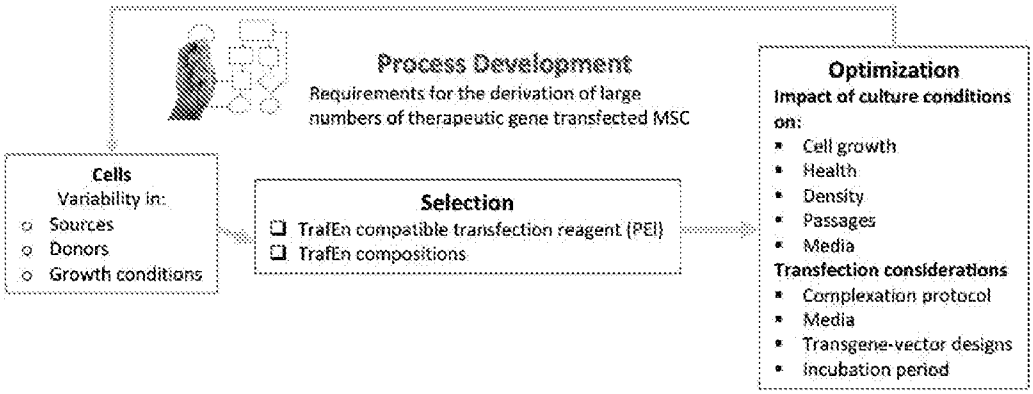

Process Development
Requirements for the derivation of large numbers of therapeutic gene transfected MSC
Cells
Variability in:
Sources
Donors
Growth conditions
Selection
TrafEn compatible transfection reagent (PEI)
TrafEn compositions
Optimization
Impact of culture conditions on:
Cell growth
Health
Density
Passages
Media
Transfection considerations
Complexation protocol
Media
Transgene-vector designs
Incubation period

FIG. 25

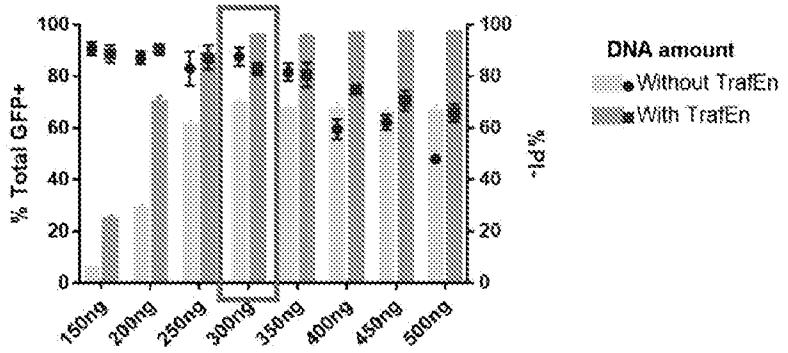

% Total GFP+
% Td+
100
80
60
40
20
0
150ng
200ng
250ng
300ng
350ng
400ng
450ng
500ng
DNA amount
Without TrafEn
With TrafEn

FIG. 26

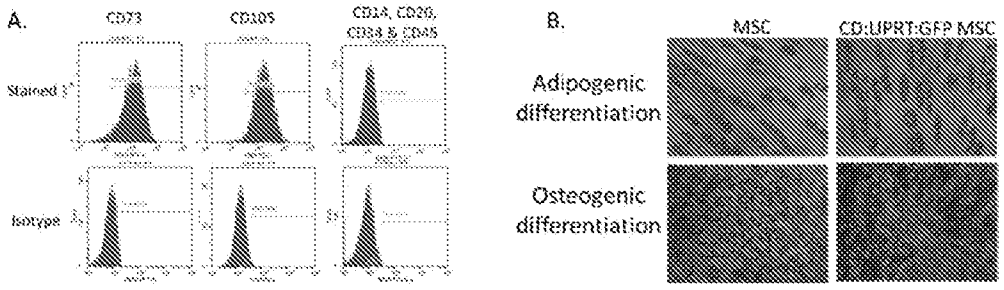


FIG. 27

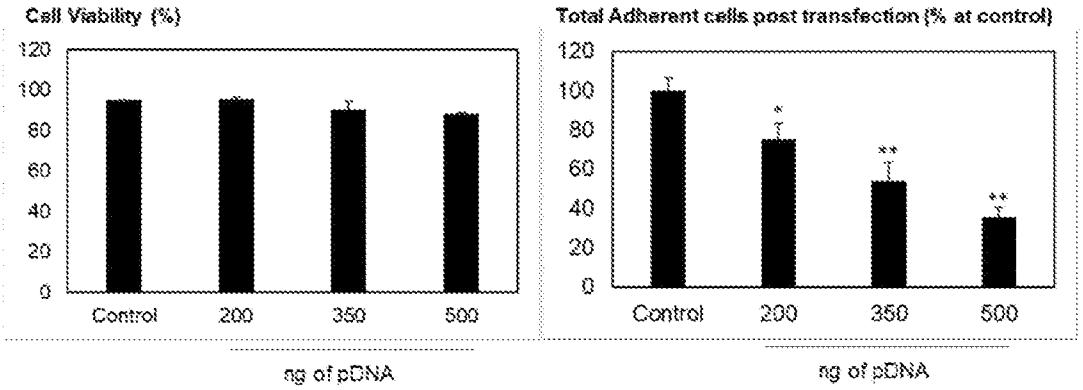


FIG. 28

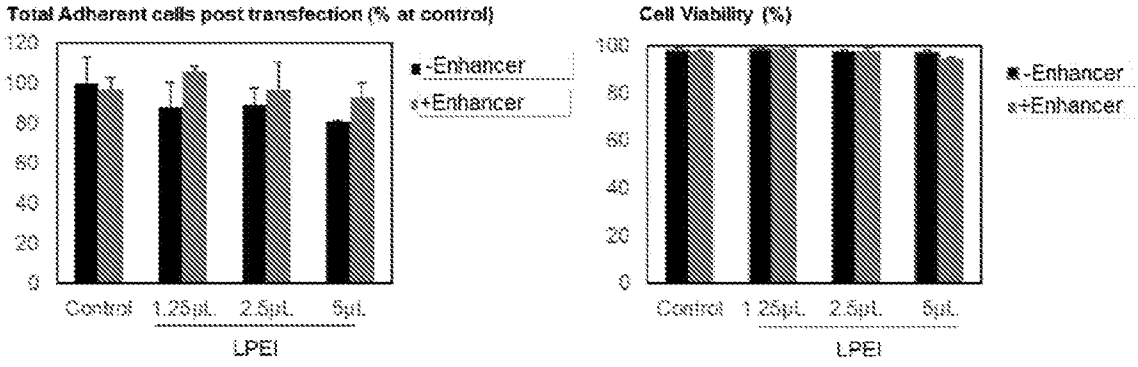


FIG. 29

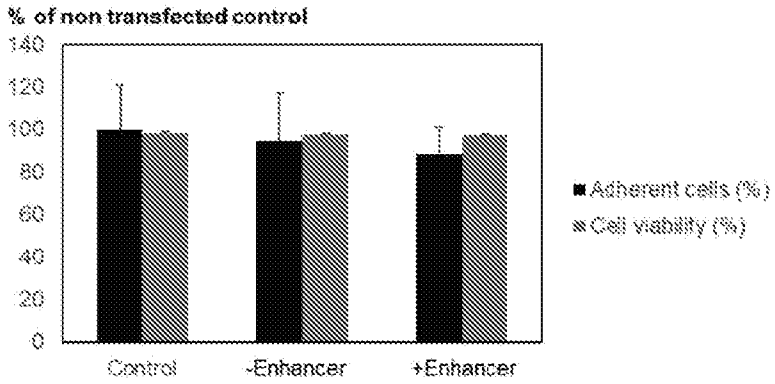

% of non transfected control
140
120
100
80
60
40
20
0
Control
-Enhancer
+Enhancer
Adherent cells (%)
Cell viability (%)

FIG. 30

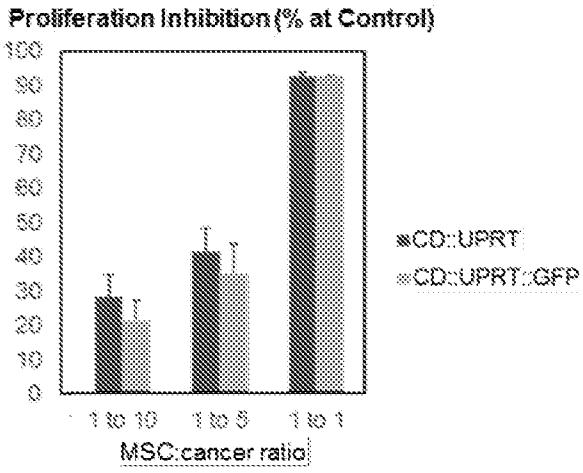

Proliferation Inhibition (% at Control)
100
90
80
70
60
50
40
30
20
10
0
1 to 10
1 to 5
1 to 1
MSC:cancer ratio
CD::UPRT
CD::UPRT::GFP

FIG. 31

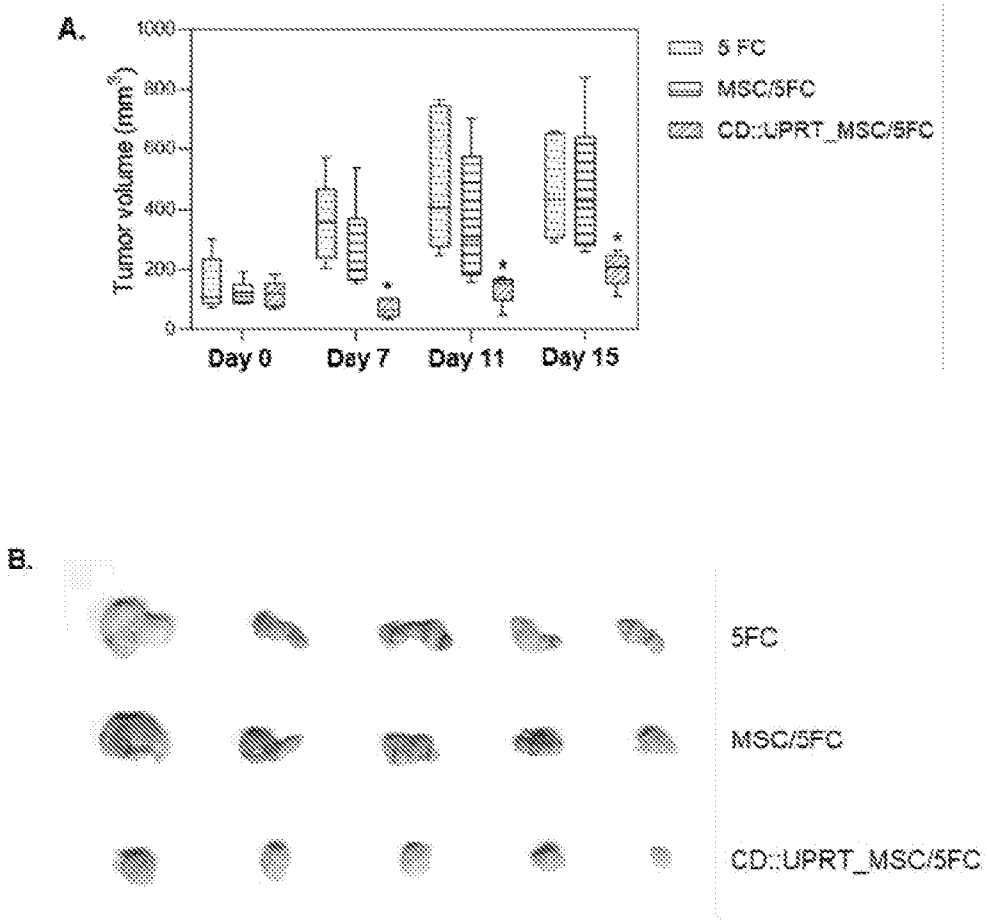

A.
1000
800
600
400
200
0
Tumor volume (mm³)
Day 0
Day 7
Day 11
Day 15
5 FC
MSC/5FC
CD::UPRT_MSC/5FC
B.
5FC
MSC/5FC
CD::UPRT_MSC/5FC

FIG. 32

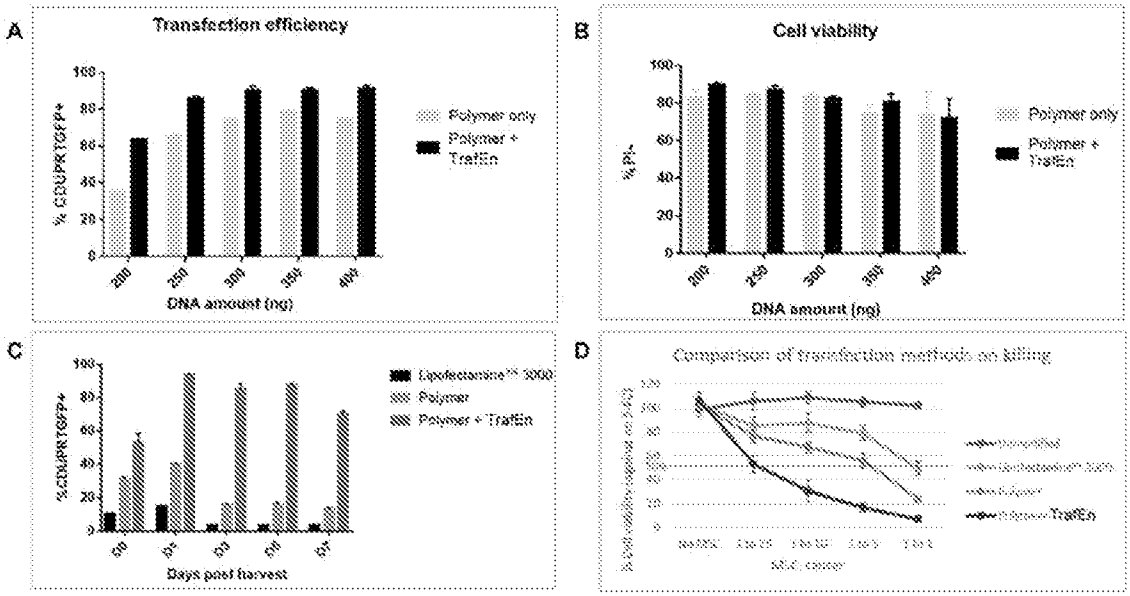

A
Transfection efficiency
% CD3/PRTGFP+
DNA amount (ng)
Polymer only
Polymer + TrafEn
B
Cell viability
% PI-
DNA amount (ng)
Polymer only
Polymer + TrafEn
C
%CD3/PRTGFP+
Days post harvest
Lipofectamine™ 3000
Polymer
Polymer + TrafEn
D
Comparison of transfection methods on killing
TrafEn

FIG. 33

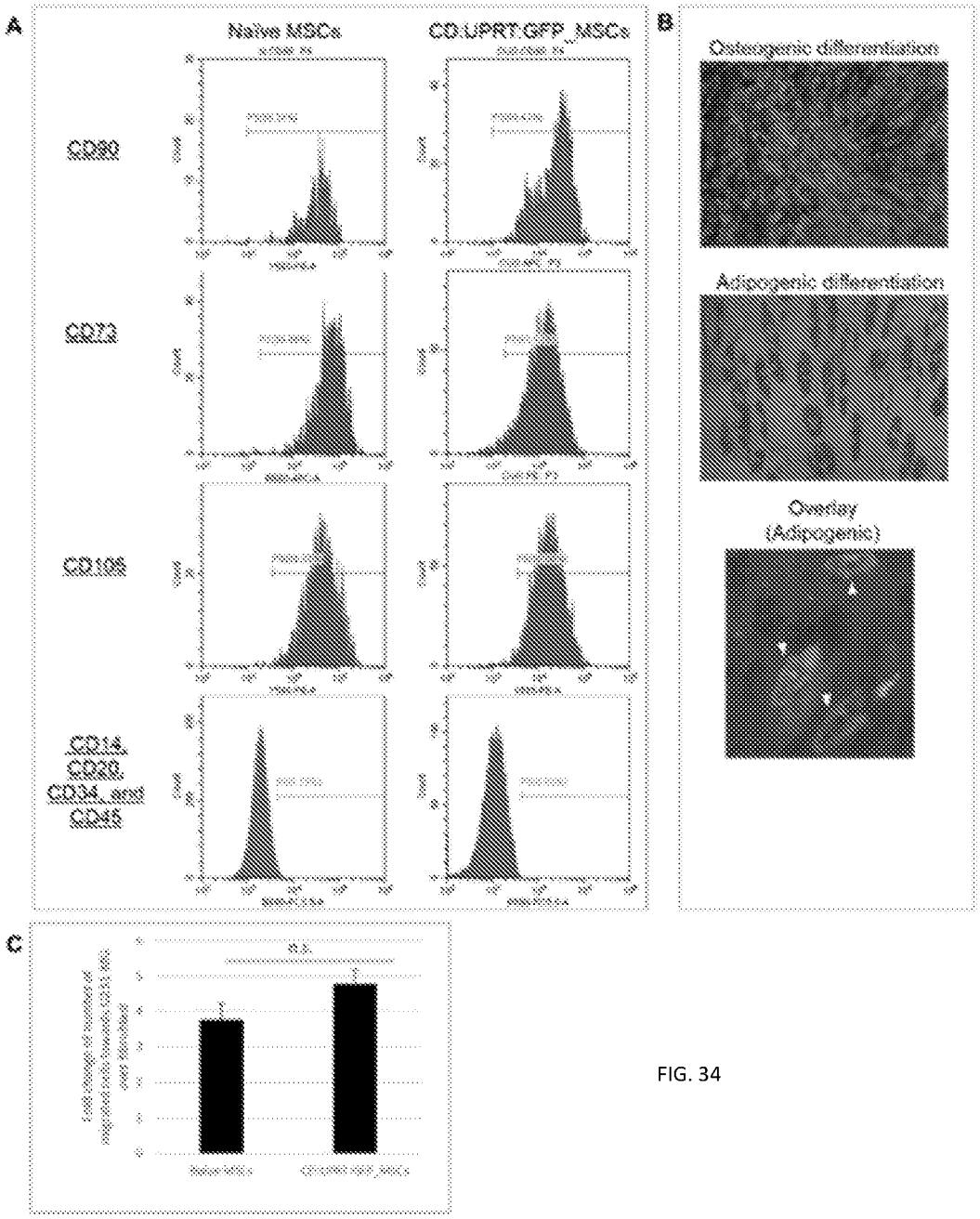

A
Naïve MSCs
CD:UPRT:GFP_MSCs
CD90
CD73
CD105
CD14, CD20, CD34, and CD45
B
Osteogenic differentiation
Adipogenic differentiation
Overlay (Adipogenic)
C

FIG. 34

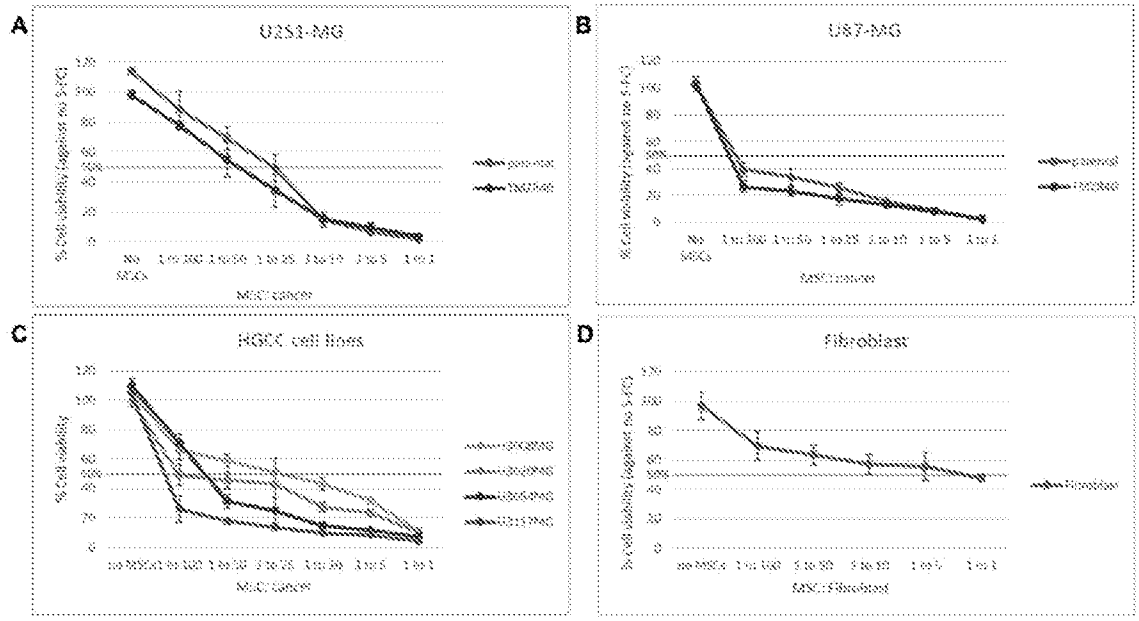


FIG. 35

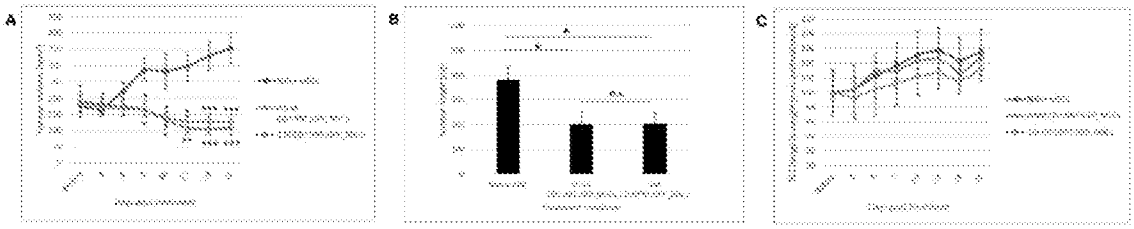


FIG. 36

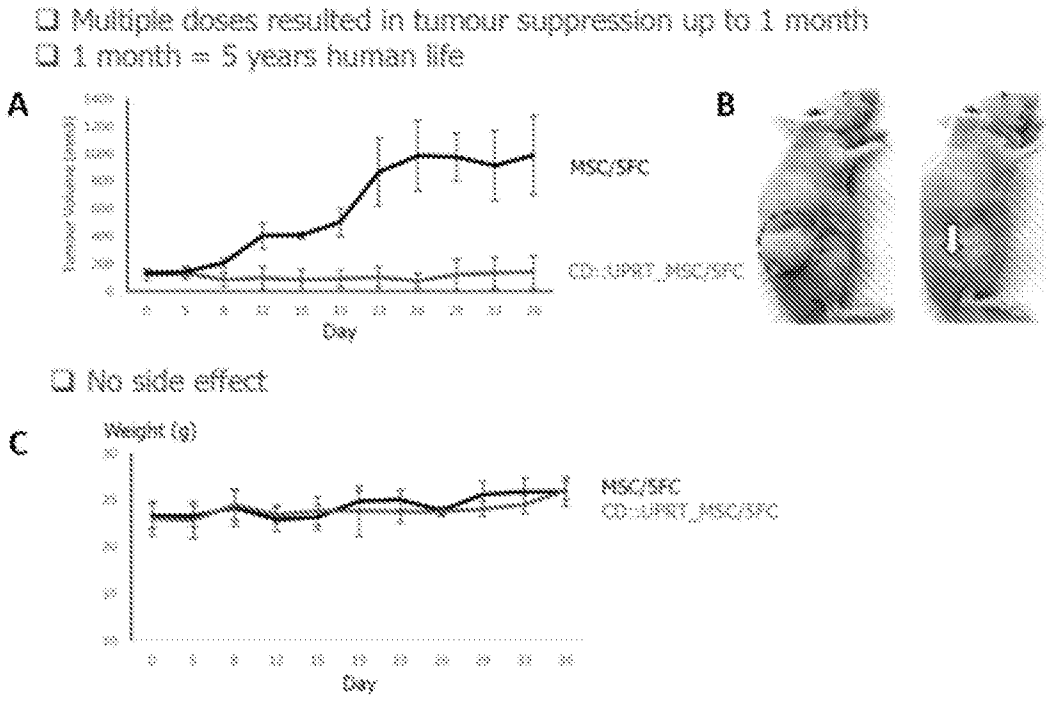

❑ Multiple doses resulted in tumour suppression up to 1 month
❑ 1 month = 5 years human life
A
B
MSC/SFC
Day
❑ No side effect
C
Weight (g)
MSC/SFC
Day

FIG. 37

modified canine MSC	2/7/2019, 9/7/2019, 16/7/2019			
	Perianal Carcinoma			
Clinical Presentation	2/7/2019	9/7/2019	16/7/2019	23/7/2019
Canine cancer patient	Before Treatment	1st Review	2nd Review	3rd Review
Weight (kg)	9	8.95	9.2	9.35
Temperature				
Tumour volume (cm^3)	2.11	2.25	3.3	3.22
Alopecia (Y/N)	N	N	N	N
Pruritis (Y/N)	N	N	N	N
Generalized Petichiae (Y/I	N	N	N	N
Vomiting (Y/N)	N	N	N	N
Diarrhea (Y/N)	N	N	N	N
Appetite (decrease?)	N	N	N	N
Activity (decrease?)	N	N	N	N
Other comments			Significant weight gain, increase fur growth and patient is less irritated by lump, does not attempt to lick/chew anymore	Increase fur growth and improved fur coat condition

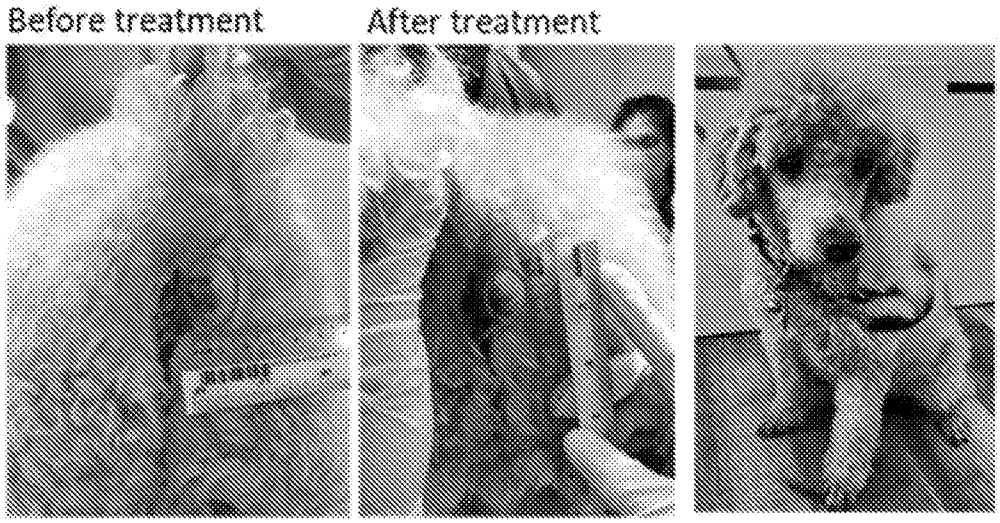


FIG. 38

Treatment Dates canine MSC administration	20/11/2019, 28/11/2019, 5/12/2019			
	Oral melanoma			
Clinical Presentation	**20/11/2019**	**28/11/2019**	**5/12/2019**	**12/12/2019**
Canine cancer patient	**Before Treatment**	**1st Review**	**2nd Review**	**3rd Review**
Weight (kg)	5.97	5.8	5.7	5.26
Temperature	37	38.4	38.1	37.8
Tumour volume (cm^3)	26.6	18.7	8.8	5.9
Alopecia (Y/N)	N	N	N	N
Pruritis (Y/N)	N	N	N	N
Generatized Petichiae (Y/N)	N	N	N	N
Vomiting (Y/N)	N	N	N	N
Diarrhea (Y/N)	N	N	Y	Y (soft stools)
Appetite (decrease?)			N (eating aggressively)	N
Activity (decrease?)		N	N	N
Other comments		More bright, alert and responsive as compared to previous visit	Same as last week. Necrotic tissue over the mass.	Distal mass was reducing in size but a proximal mass appearing.

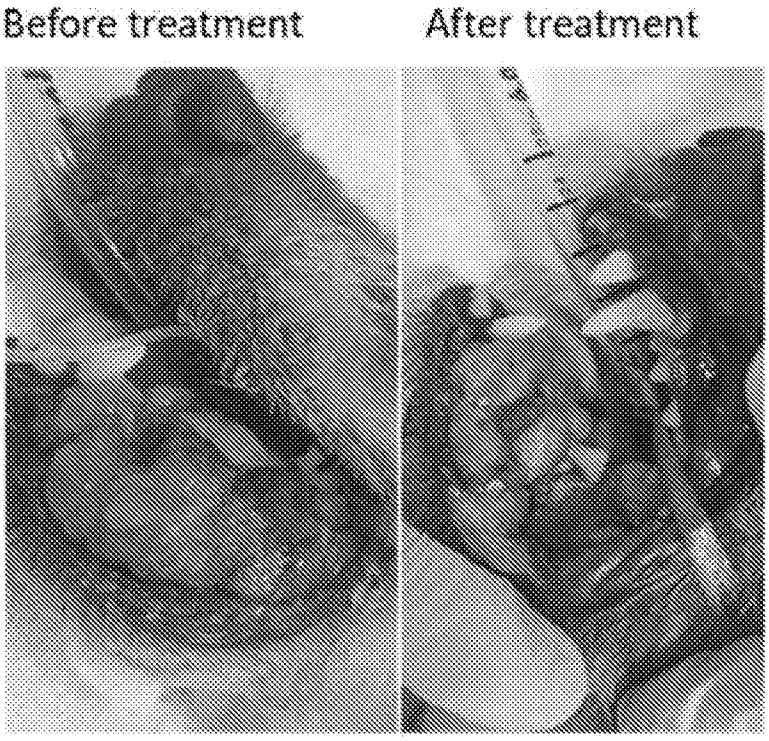


FIG. 39

Treatment Dates canine MSC administration	28/08/2018, 4/09/2018, 11/09/2018			
	Thyroid carcinoma			
Clinical Presentation	28/8/2018	4/9/2018	11/9/2018	20/9/2018
Canine cancer patient	Before Treatment	1st Review	2nd Review	3rd Review
Weight (kg)	20.4	21.05	20.8	20.65
Tumour volume (cm^3)	288	239	225	230
Alopecia (Y/N)	N	N	N	N
Pruritis (Y/N)	N	N	N	N
Generalized Petichiae (Y/N)	N	N	N	N
Vomiting (Y/N)	N	N	N	N
Diarrhea (Y/N)	N	N	N	N
Appetite (decrease?)	Good Appetite	Lesser	Good Appetite	Good Appetite
Other comments		Tumor feels harder. Breathing difficulty	Tumor is harder. Breathing improved	Breathing difficulty but not worse than previous. No problem swallowing.

FIG. 40

Treatment Dates canine MSC administration	05/10/2018, 11/10/2018, 17/10/2018			
	Soft tissue sarcoma			
Clinical Presentation	5/10/2018	11/10/2018	17/10/2018	25/9/2018
Canine cancer patient	Before Treatment	1st Review	2nd Review	3rd Review
Weight (kg)	7.3	7.4	7.9	7.45
Tumour volume (cm^3)	2.3	2.6	2.6	0.4
Alopecia (Y/N)	N	N	N	N
Pruritis (Y/N)	N	N	N	N
Generalized Petichiae (Y/N)	N	N	N	N
Vomiting (Y/N)	N	N	N	N
Diarrhea (Y/N)	N	N	N	N
Appetite (decrease?)	Good Appetite	Good Appetite	Good Appetite	Good Appetite
Other comments	More active and better appetite	Reduced inflammation		Scab formation tumor mass not observed

Before treatment After treatment

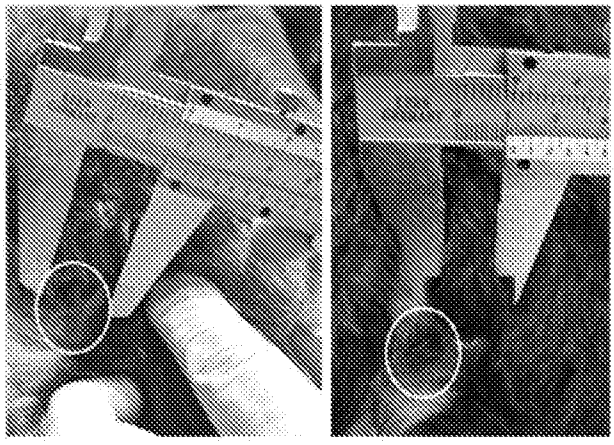


FIG. 41

	Before treatment	After first dose	After second dose
	30/11/2019	6/12/19	13/12/2019
Length	43	36	30
Width	36	33	34.9
Height	30	20	20

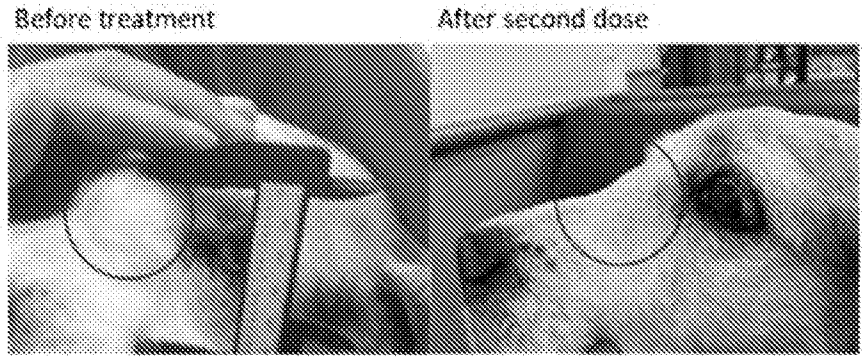


FIG. 42

Treatment Dates				
canine MSC administration	31/05/2019, 06/06/2019, 21/06/2019			
	gastrointestinal cancer			
Clinical Presentation	5/10/2018	11/10/2018	17/10/2018	25/9/2018
Canine cancer patient	Before Treatment	1st Review	2nd Review	3rd Review
Weight (kg)	29.2	29.1	29.15	29.35
Alopecia (Y/N)	N	N	N	N
Pruritis (Y/N)	N	N	N	N
Generalized Petichiae (Y/N)	N	N	N	N
Vomiting (Y/N)	N	N	N	N
Diarrhea (Y/N)	N	N	N	N
Appetite (decrease?)	N	N	N	N
Other comments	throw up after infusion, no fever			

Ultrasound report

Masses	Before 30-5-2019	After 9-7-2019
Pancreas	1.7mm	Appear normal
Pancreas	1.7mm	Appear normal
Hypoechoic mass	38.7 x 24.2mm	10 x 9 mm

FIG. 43

❑ Validated in >12 donors (age

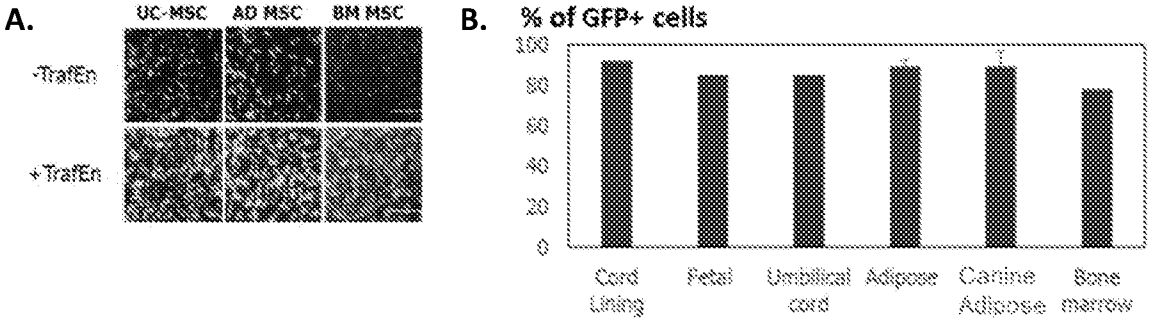


FIG. 44

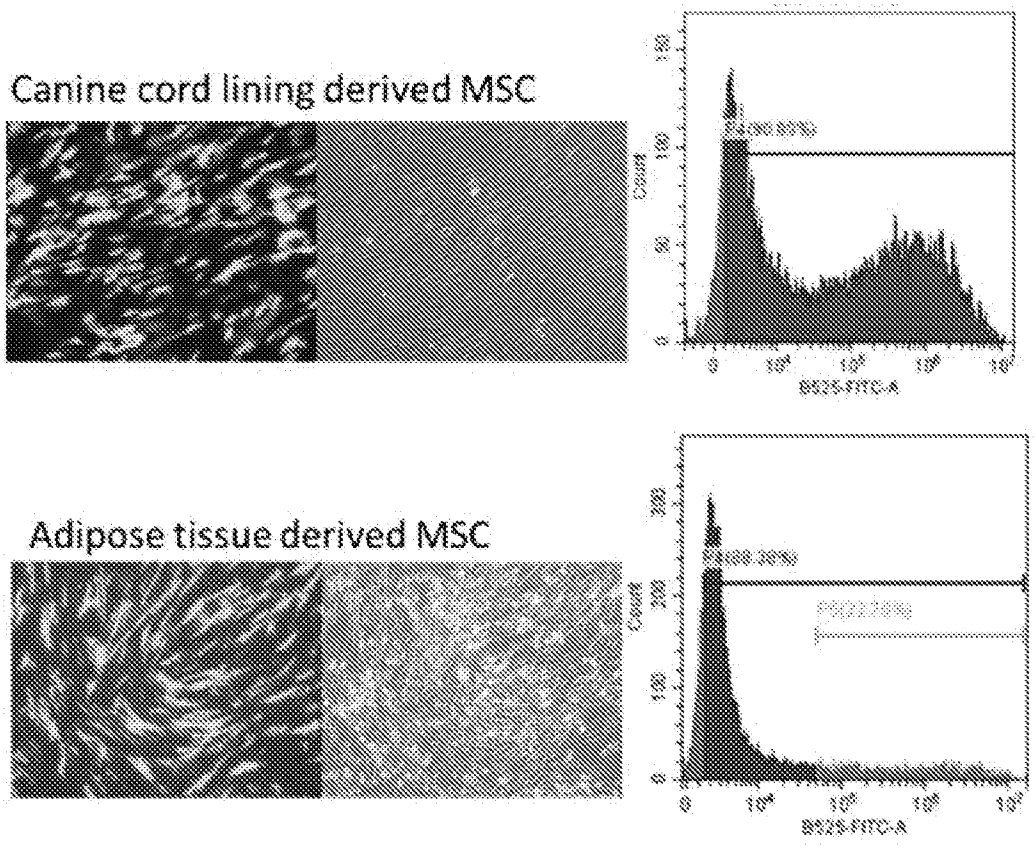


FIG. 45

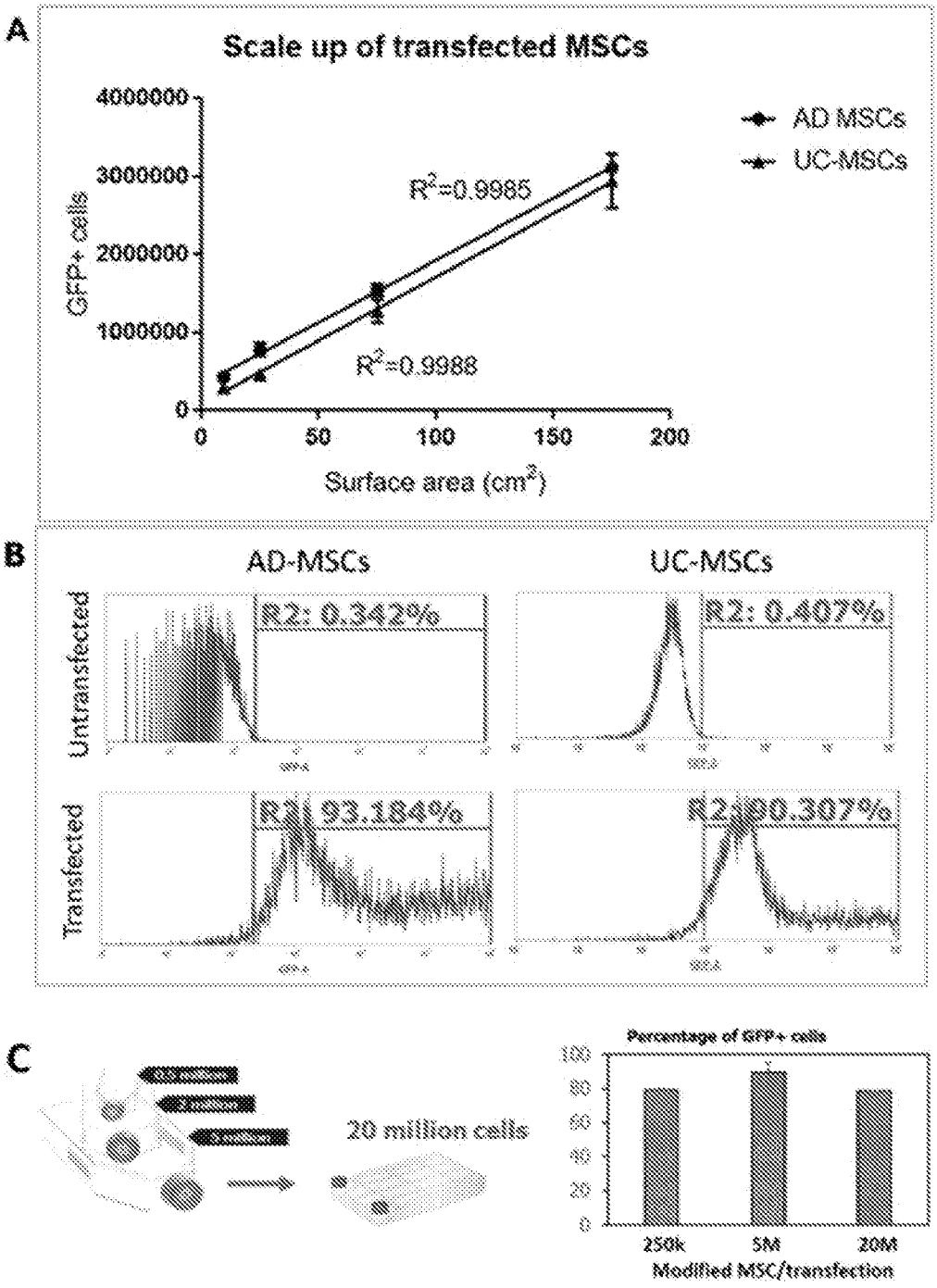

A
Scale up of transfected MSCs
AD MSCs
UC-MSCs
GFP+ cells
4000000
3000000
2000000
1000000
0
R2=0.9985
R2=0.9988
0
50
100
150
200
Surface area (cm2)
B
AD-MSCs
UC-MSCs
Untransfected
Transfected
R2: 0.342%
R2: 0.407%
R2: 93.184%
R2: 90.307%
C
20 million cells
Percentage of GFP+ cells
100
80
60
40
20
0
250k
5M
20M
Modified MSC/transfection

FIG. 46

A

Microcarrier	Coating	Charge
C-GEN 102	Type I porcine collagen	No charge
Pro-F 102	Proherin® S, a recombinant	No charge
P Plus 102 L	Uncoated	Positive
Cytodex® 1	Uncoated	Positive
Cytodex® 3	Type I porcine collagen	No charge
Synthemax® II	Proprietary synthetic peptide acrylate	NA

B

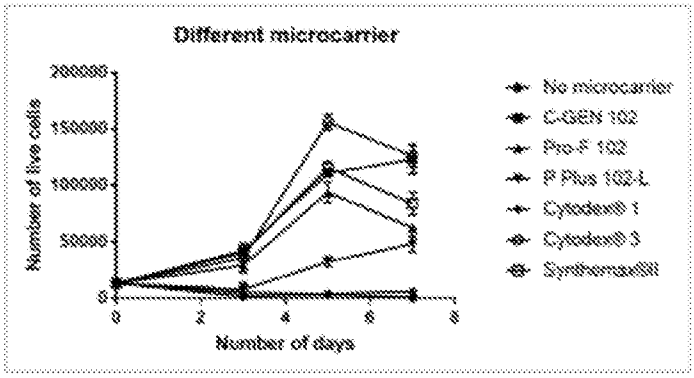
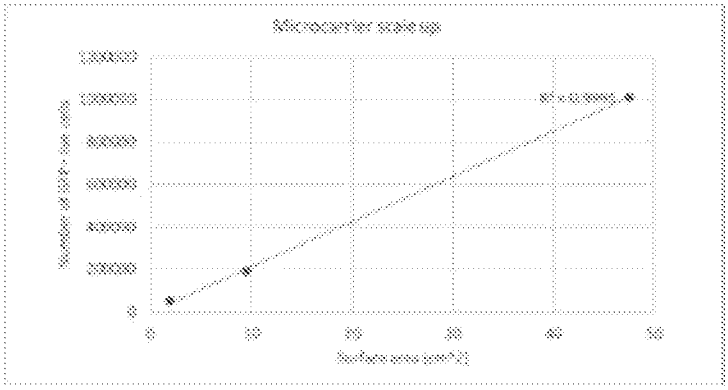


FIG. 47

A



B

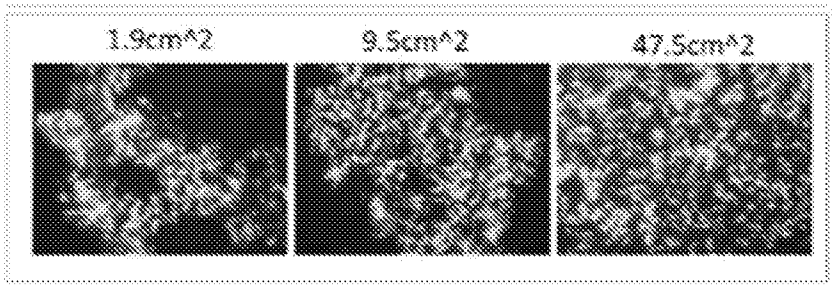


FIG. 48

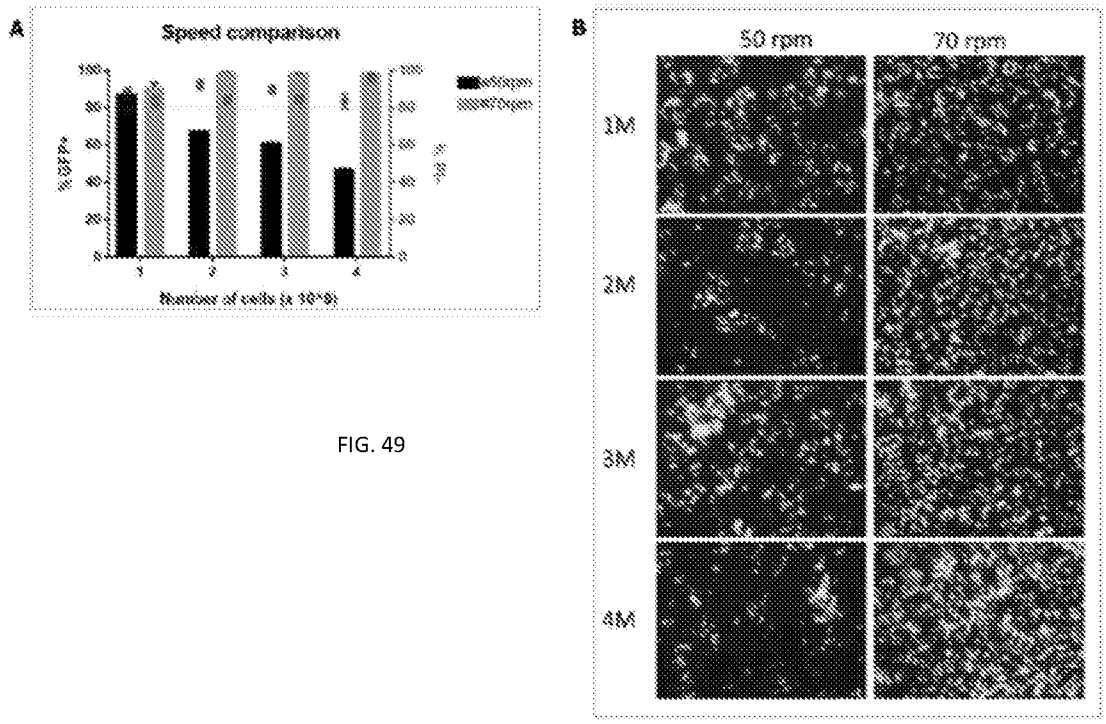

A
Speed comparison
B
50 rpm
70 rpm
1M
2M
3M
4M

FIG. 49

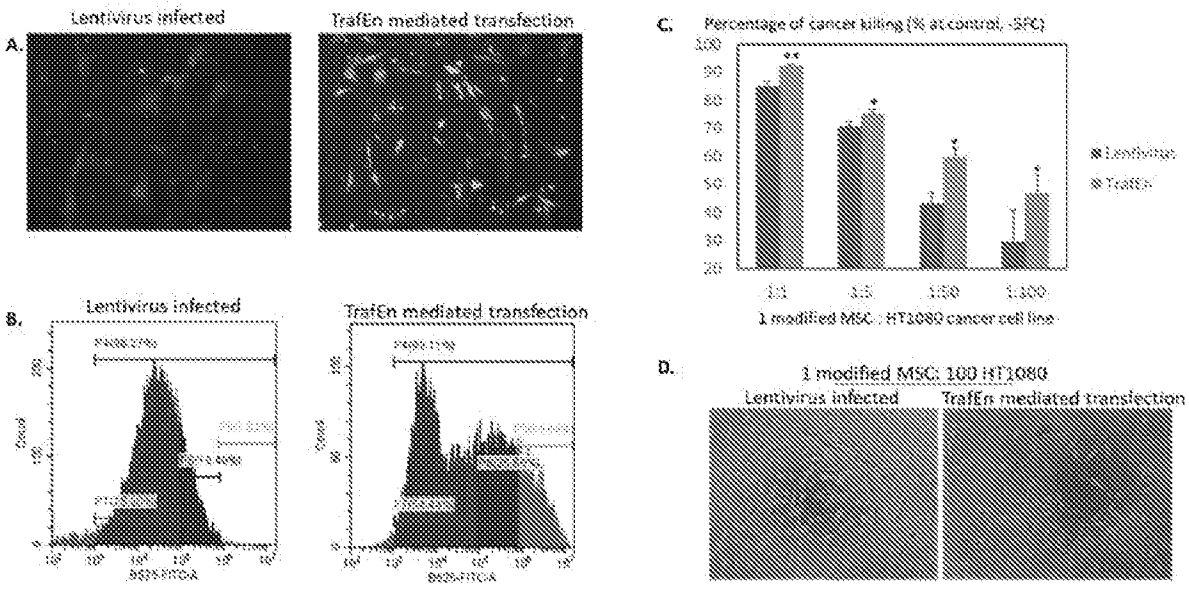

A.
Lentivirus infected
TrafEn mediated transfection
B.
Lentivirus infected
TrafEn mediated transfection
C.
Percentage of cancer killing (% at control, -SFC)
1 modified MSC : HT1080 cancer cell line
D.
1 modified MSC: 100 HT1080
Lentivirus infected
TrafEn mediated transfection

FIG. 50

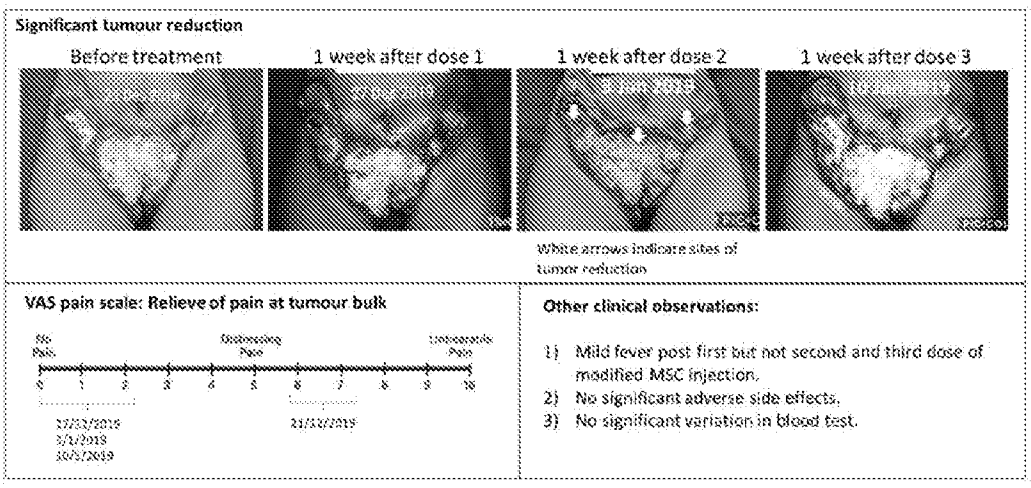

Significant tumour reduction
Before treatment
1 week after dose 1
1 week after dose 2
1 week after dose 3
White arrows indicate sites of tumor reduction
VAS pain scale: Relieve of pain at tumour bulk
No Pain
Unbearable Pain
0 1 2 3 4 5 6 7 8 9 10
Other clinical observations:
1) Mild fever post first but not second and third dose of modified MSC injection.
2) No significant adverse side effects.
3) No significant variation in blood test.

FIG. 51

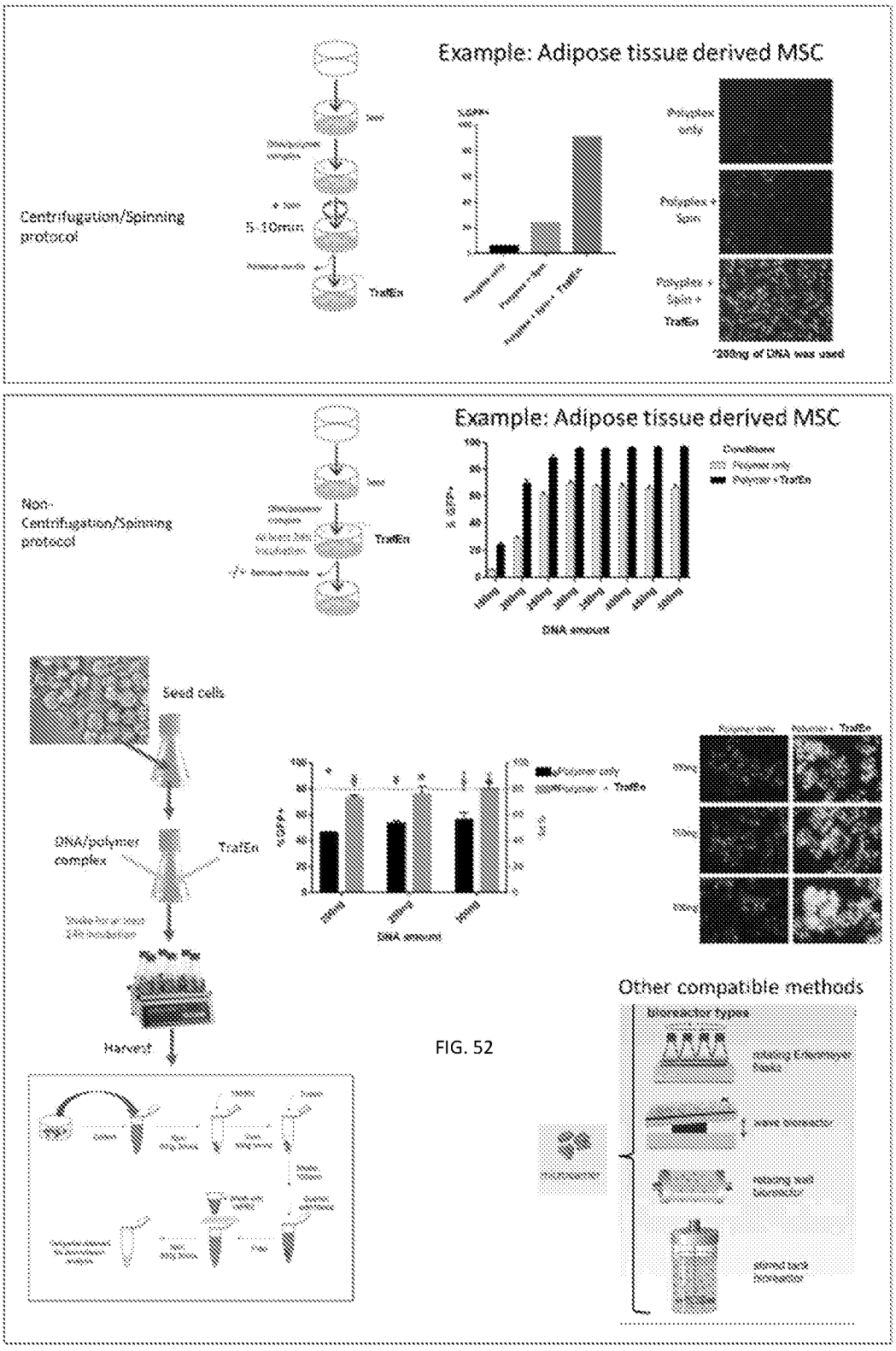

Example: Adipose tissue derived MSC
Centrifugation/Spinning protocol
5-10min
TrafEn
Example: Adipose tissue derived MSC
Non-Centrifugation/Spinning protocol
TrafEn
DNA amount
Seed cells
DNA/polymer complex
TrafEn
Harvest
DNA amount
Other compatible methods
Bioreactor types

FIG. 52

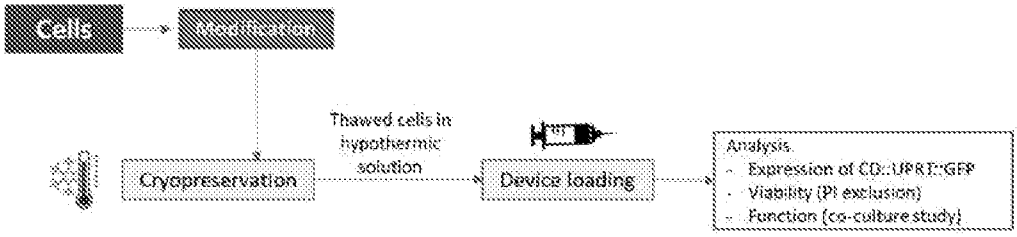

Cells
Modification
Cryopreservation
Thawed cells in hypothermic solution
Device loading
Analysis
- Expression of CD::UPRT::GFP
- Viability (PI exclusion)
- Function (co-culture study)

FIG. 53

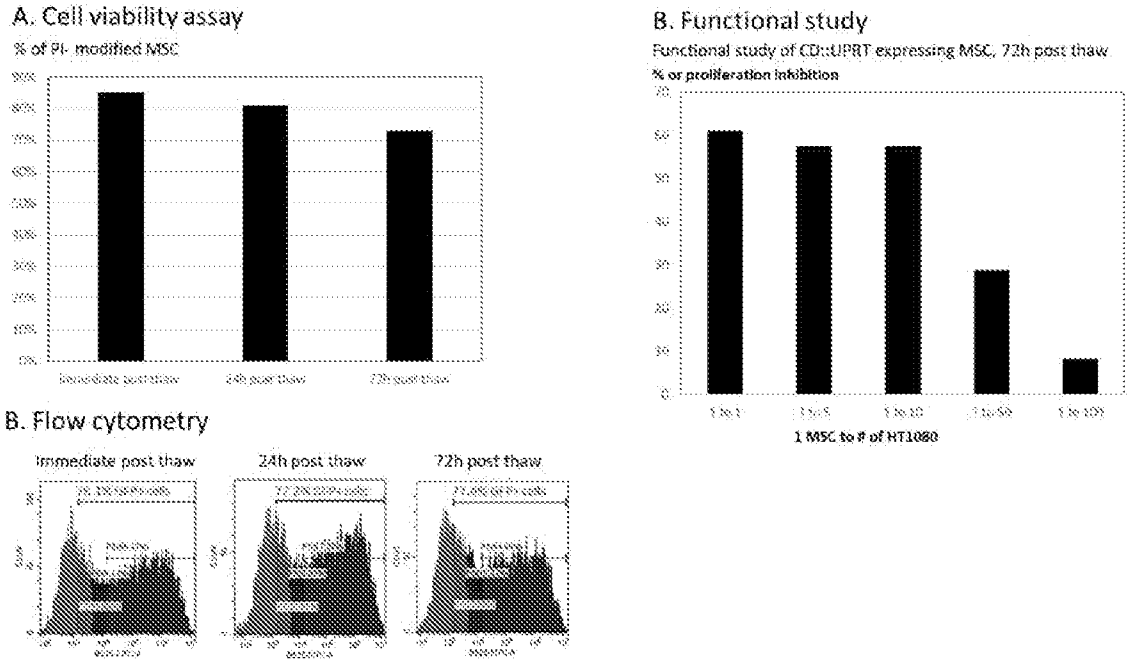

A. Cell viability assay
% of PI- modified MSC
B. Flow cytometry
Immediate post thaw
24h post thaw
72h post thaw
B. Functional study
Functional study of CD::UPRT expressing MSC, 72h post thaw
% or proliferation inhibition
1 MSC to # of HT1080

FIG. 54