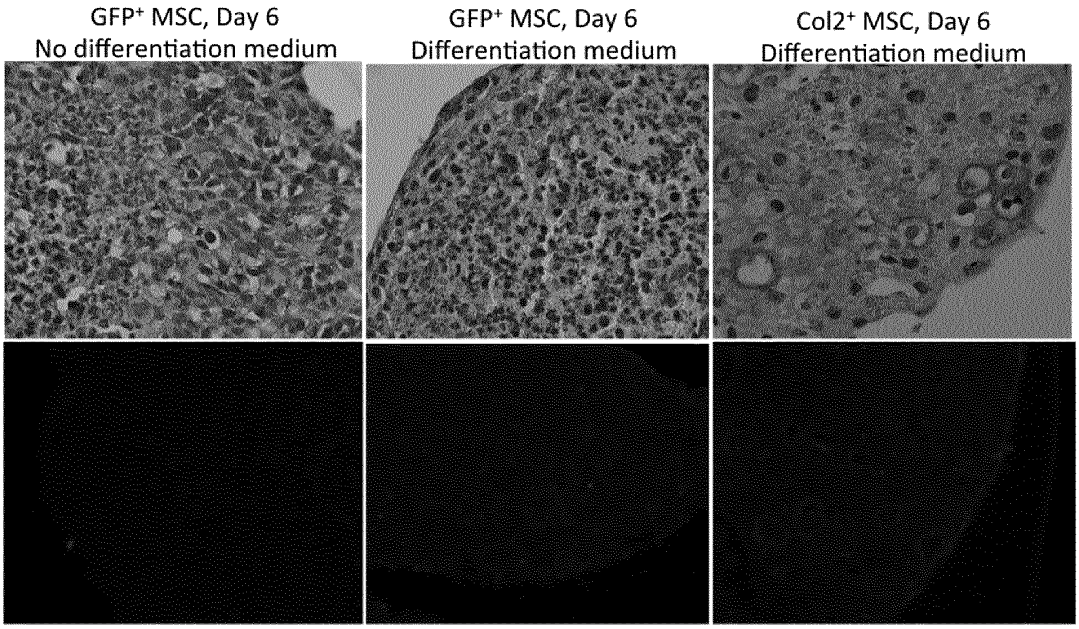

GFP+ MSC, Day 6
No differentiation medium
GFP+ MSC, Day 6
Differentiation medium
Col2+ MSC, Day 6
Differentiation medium
Alcian blue
IHC against type X collagen, 40X

FIG.1

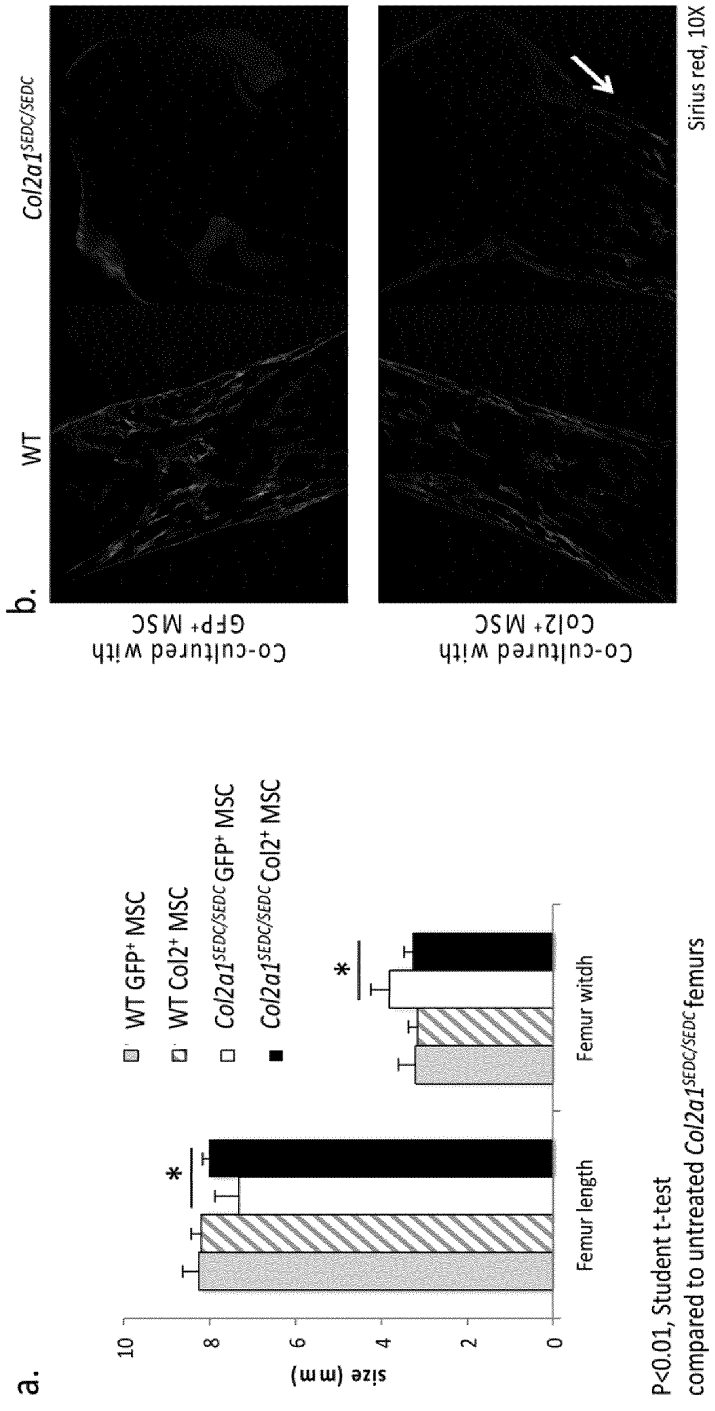

b.
WT
Col2a1SEDC/SEDC
Co-cultured with GFP+ MSC
Co-cultured with Col2+ MSC
Sirius red, 10X
a.
WT GFP+ MSC
WT Col2+ MSC
Col2a1SEDC/SEDC GFP+ MSC
Col2a1SEDC/SEDC Col2+ MSC
size (mm)
0
2
4
6
8
10
*
*
Femur length
Femur witdh
P<0.01, Student t-test
compared to untreated Col2a1SEDC/SEDC femurs

FIG.2

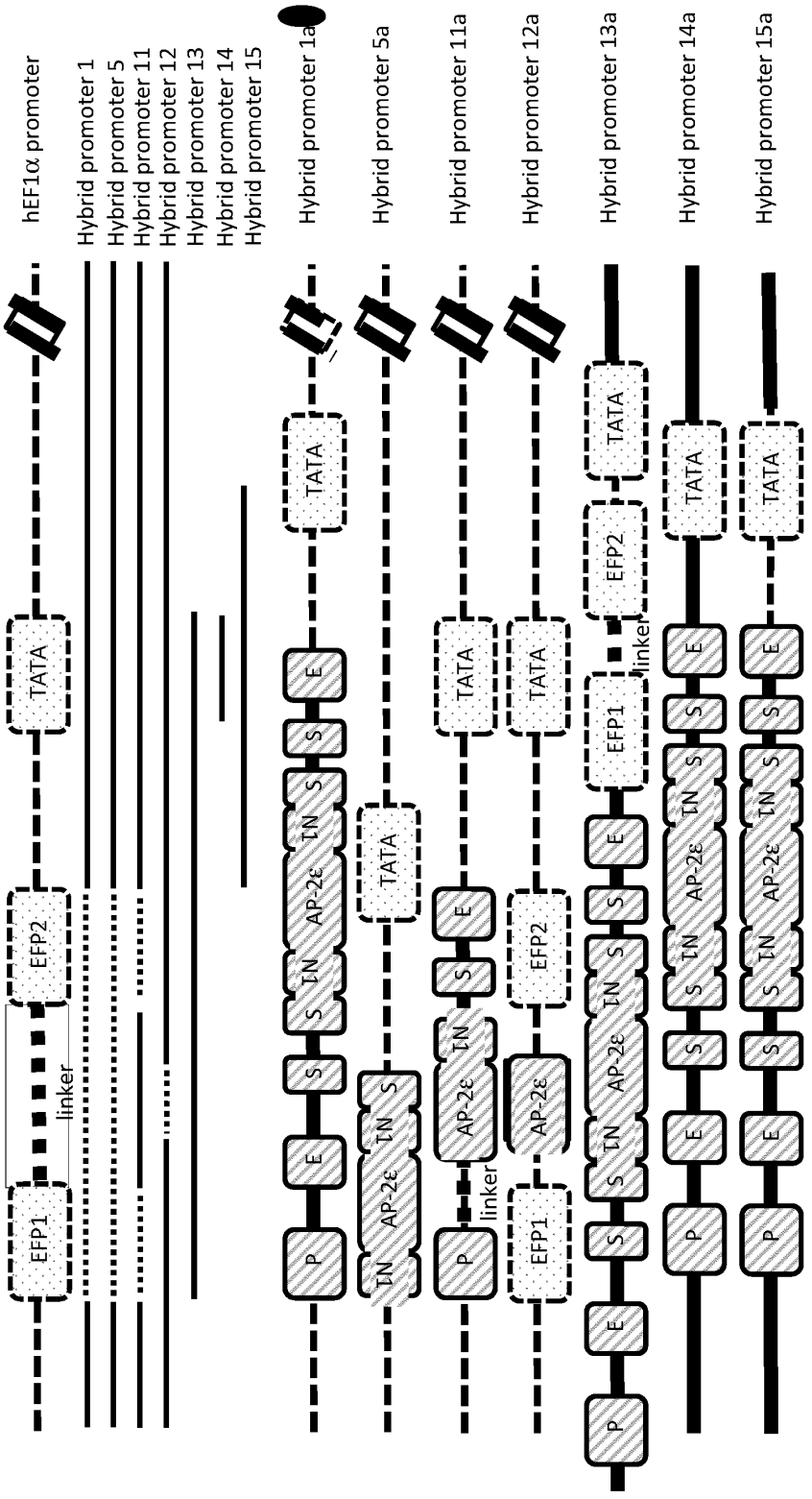

hEF1α promoter
Hybrid promoter 1
Hybrid promoter 5
Hybrid promoter 11
Hybrid promoter 12
Hybrid promoter 13
Hybrid promoter 14
Hybrid promoter 15
Hybrid promoter 1a
Hybrid promoter 5a
Hybrid promoter 11a
Hybrid promoter 12a
Hybrid promoter 13a
Hybrid promoter 14a
Hybrid promoter 15a
TATA
EFP2
EFP1
linker
AP-2ε
TN
S
E
P

FIG.3

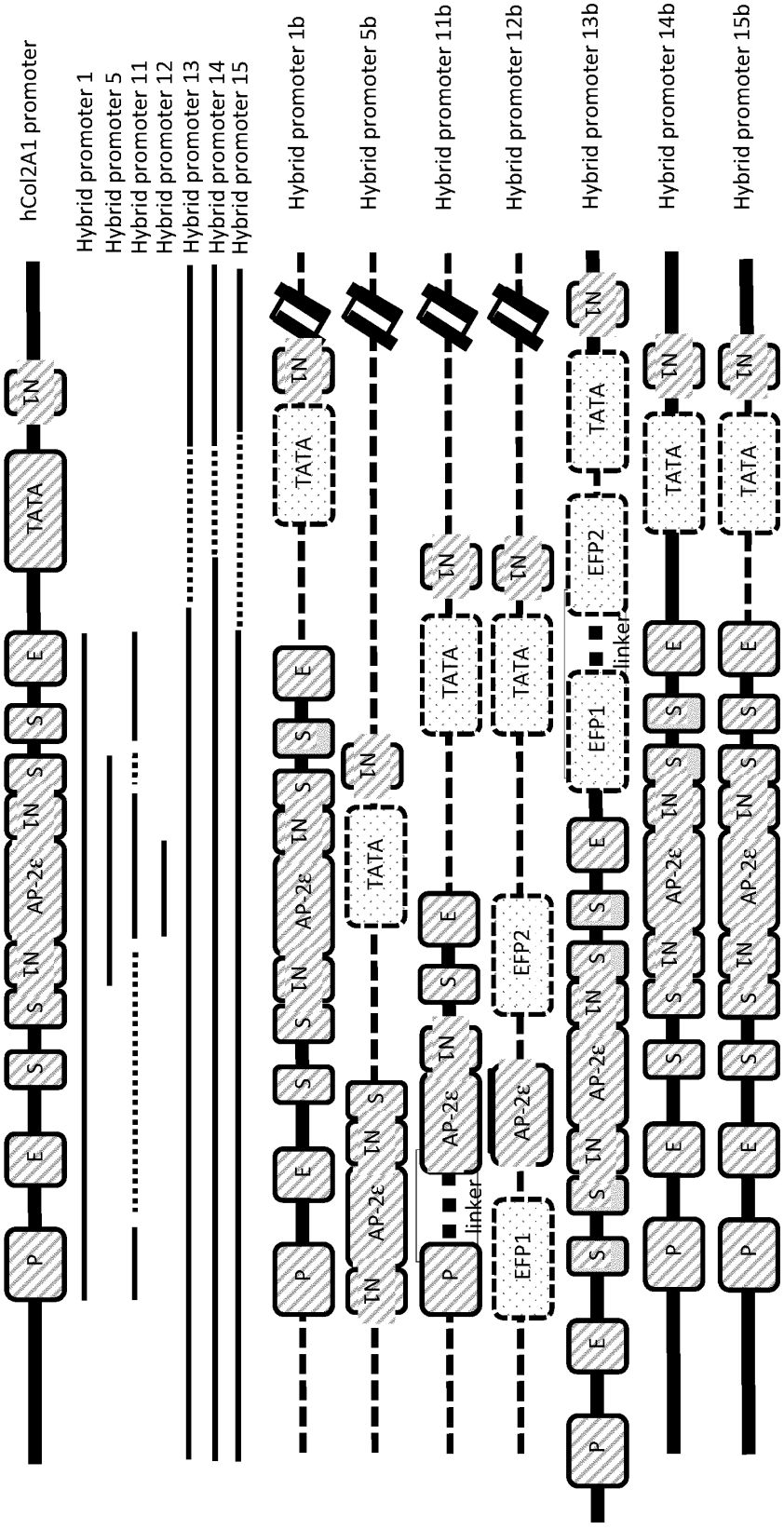

hCol2A1 promoter
Hybrid promoter 1
Hybrid promoter 5
Hybrid promoter 11
Hybrid promoter 12
Hybrid promoter 13
Hybrid promoter 14
Hybrid promoter 15
Hybrid promoter 1b
Hybrid promoter 5b
Hybrid promoter 11b
Hybrid promoter 12b
Hybrid promoter 13b
Hybrid promoter 14b
Hybrid promoter 15b
P
E
S
IN
AP-2ε
TATA
EFP1
EFP2
linker

FIG.4

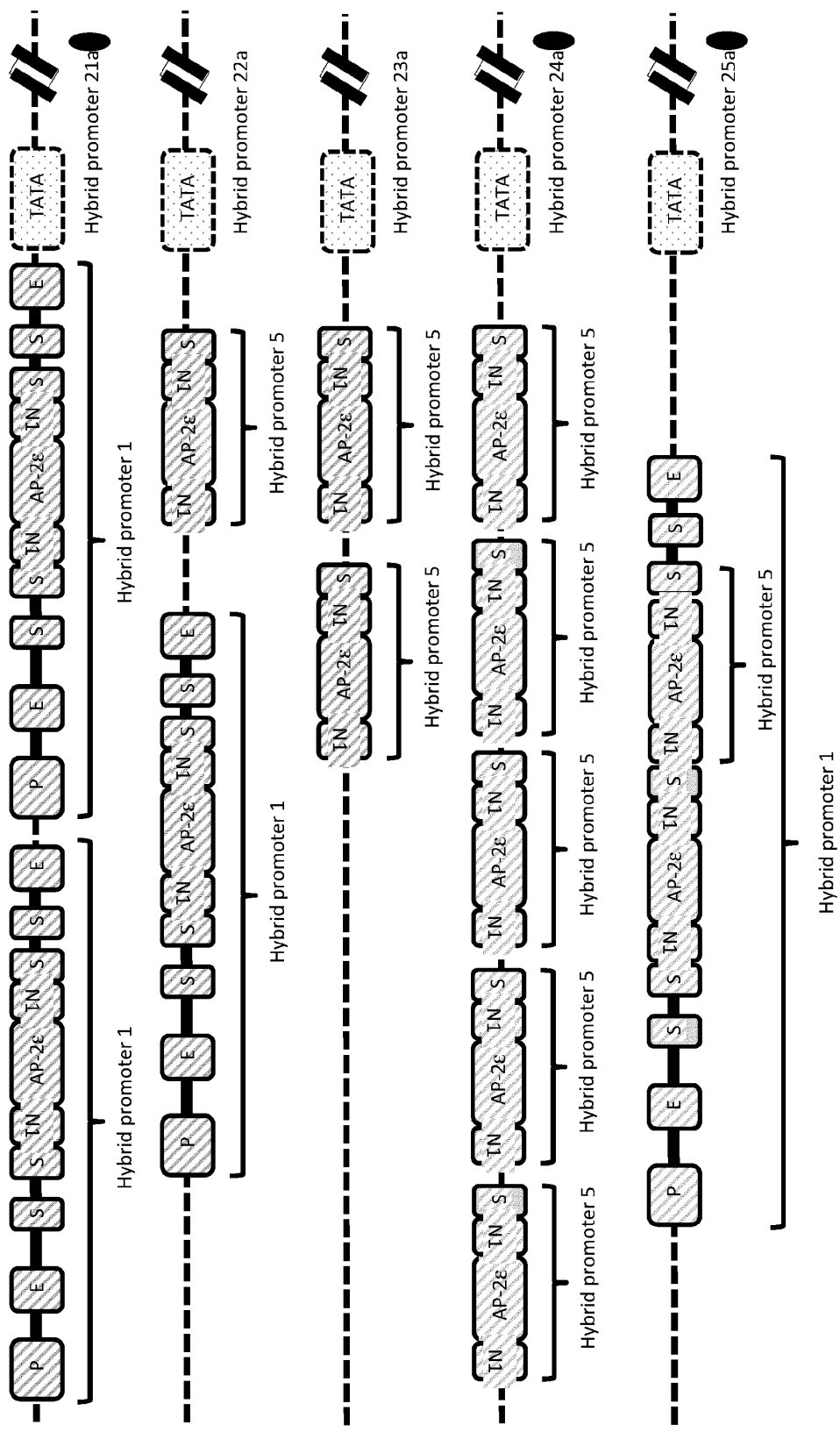

P
E
S
S
TN
AP-2ε
TN
S
S
E
P
E
S
S
TN
AP-2ε
TN
S
S
E
P
E
S
S
TN
AP-2ε
TN
S
S
E
TATA
Hybrid promoter 21a
Hybrid promoter 1
Hybrid promoter 22a
Hybrid promoter 5
Hybrid promoter 1
Hybrid promoter 23a
Hybrid promoter 5
Hybrid promoter 5
Hybrid promoter 24a
Hybrid promoter 5
Hybrid promoter 5
Hybrid promoter 5
Hybrid promoter 25a
Hybrid promoter 5
Hybrid promoter 1

FIG.5

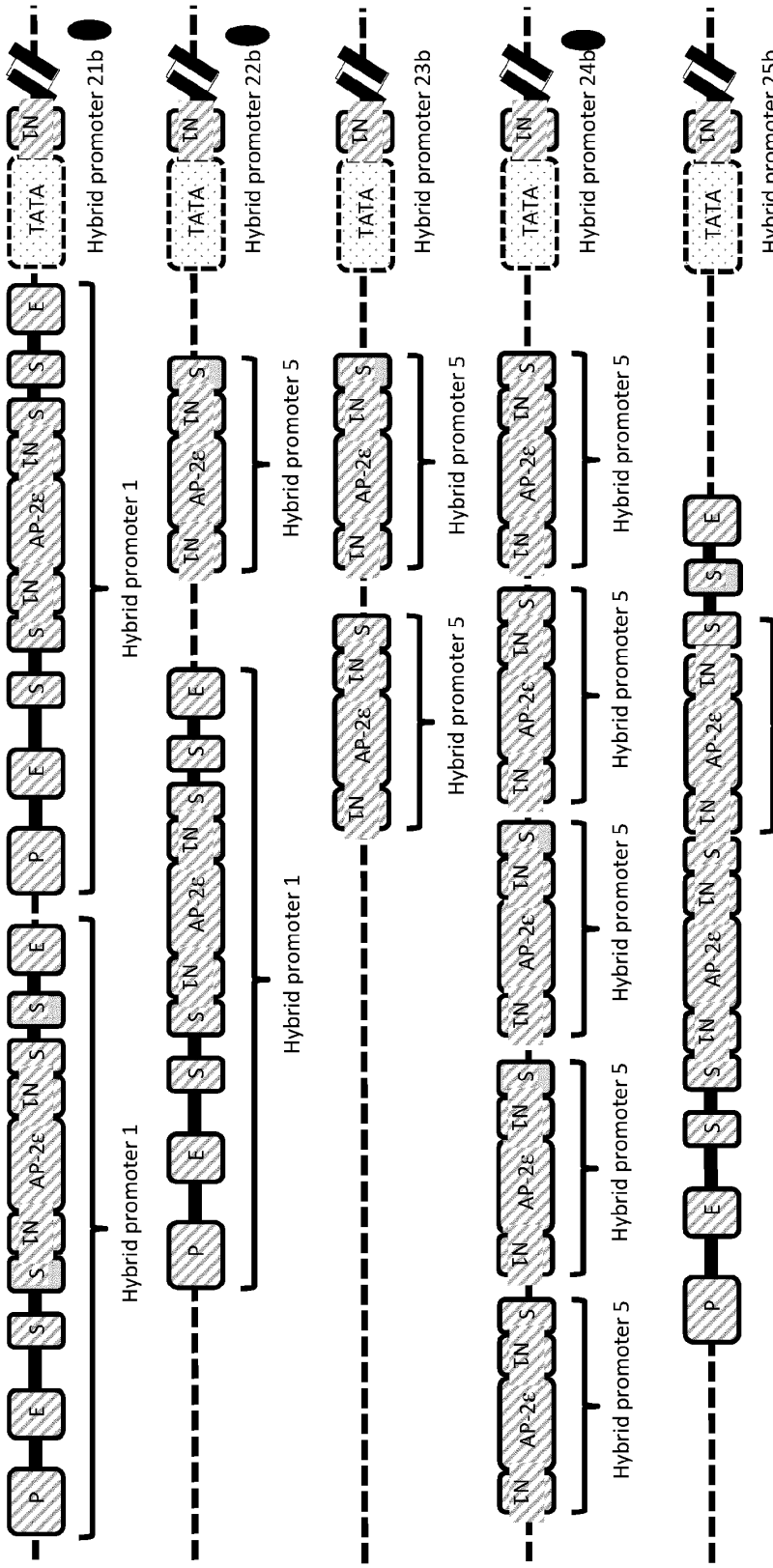

P
E
S
S
TN
AP-2ε
TN
S
S
E
P
E
S
S
TN
AP-2ε
TN
S
S
E
TATA
TN
Hybrid promoter 1
Hybrid promoter 21b
Hybrid promoter 5
Hybrid promoter 22b
Hybrid promoter 23b
Hybrid promoter 24b
Hybrid promoter 25b

FIG.6

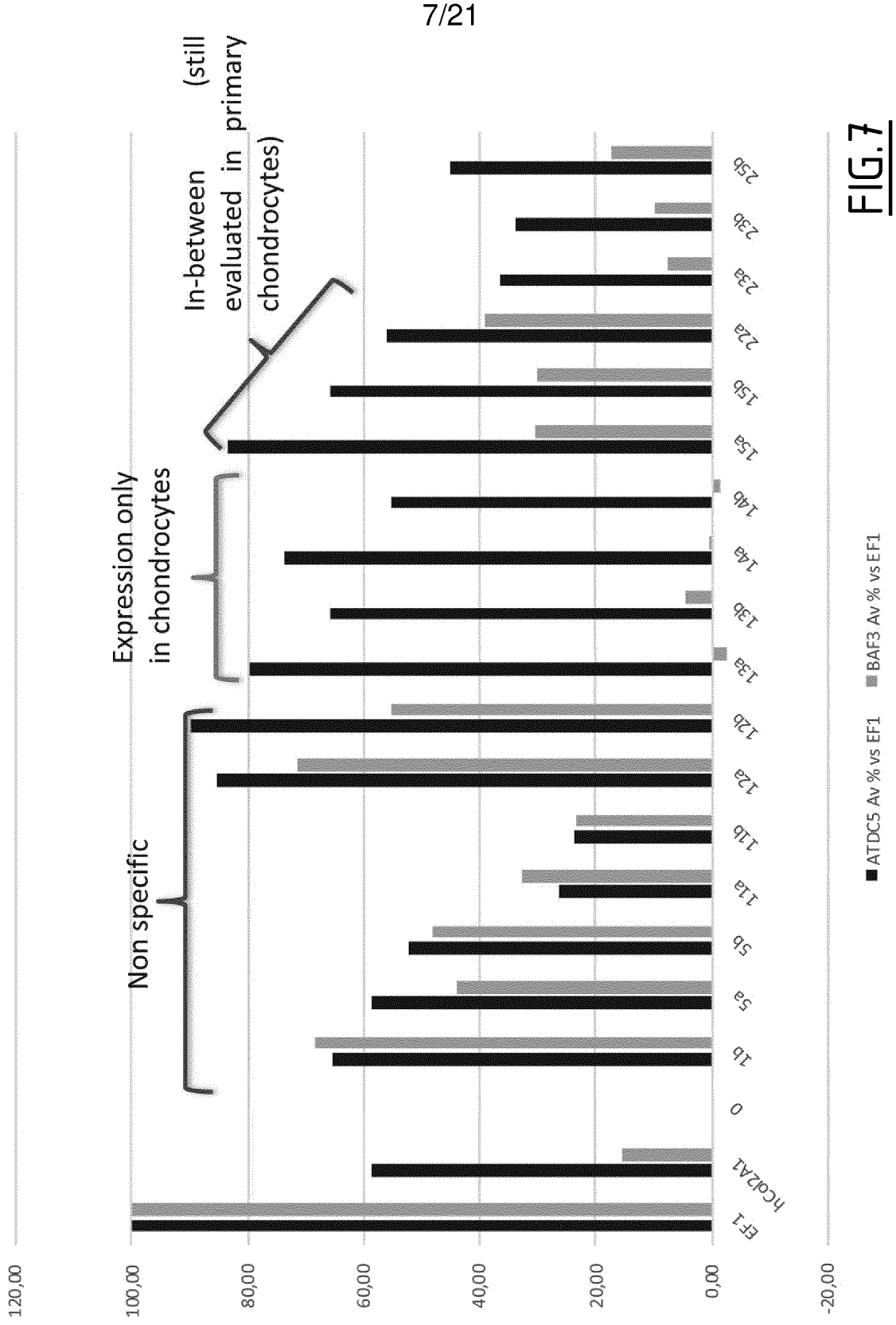

Non specific
Expression only in chondrocytes
In-between (still evaluated in primary chondrocytes)
EF1
hcol2A1
0
1b
5a
5b
11a
11b
12a
12b
13a
13b
14a
14b
15a
15b
22a
23a
23b
25b
120,00
100,00
80,00
60,00
40,00
20,00
0,00
-20,00
ATDC5 Av % vs EF1
BAF3 Av % vs EF1

FIG.7

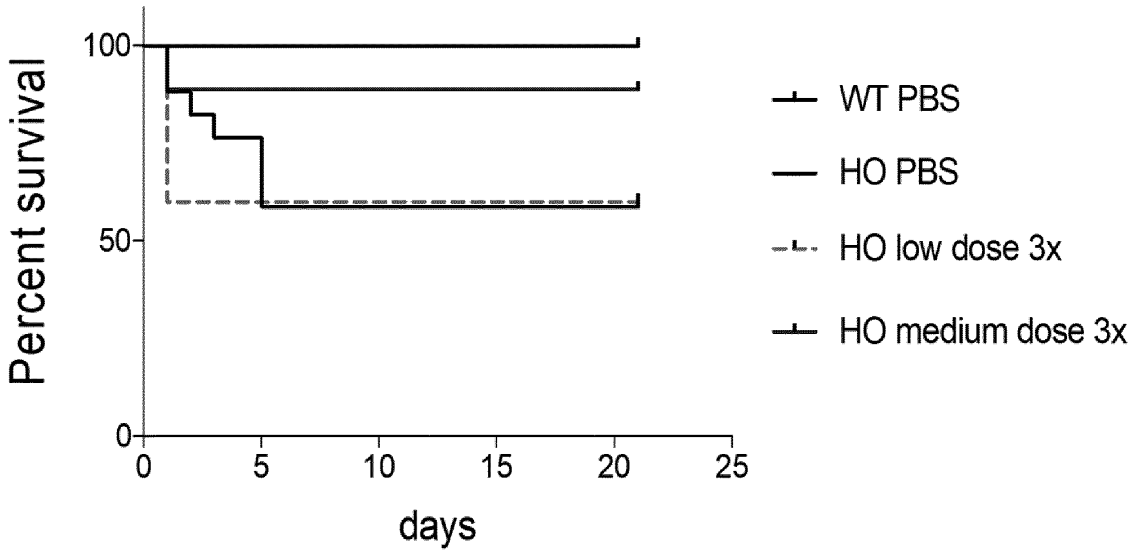

Percent survival
100
50
0
0
5
10
15
20
25
days
WT PBS
HO PBS
HO low dose 3x
HO medium dose 3x

FIG.8

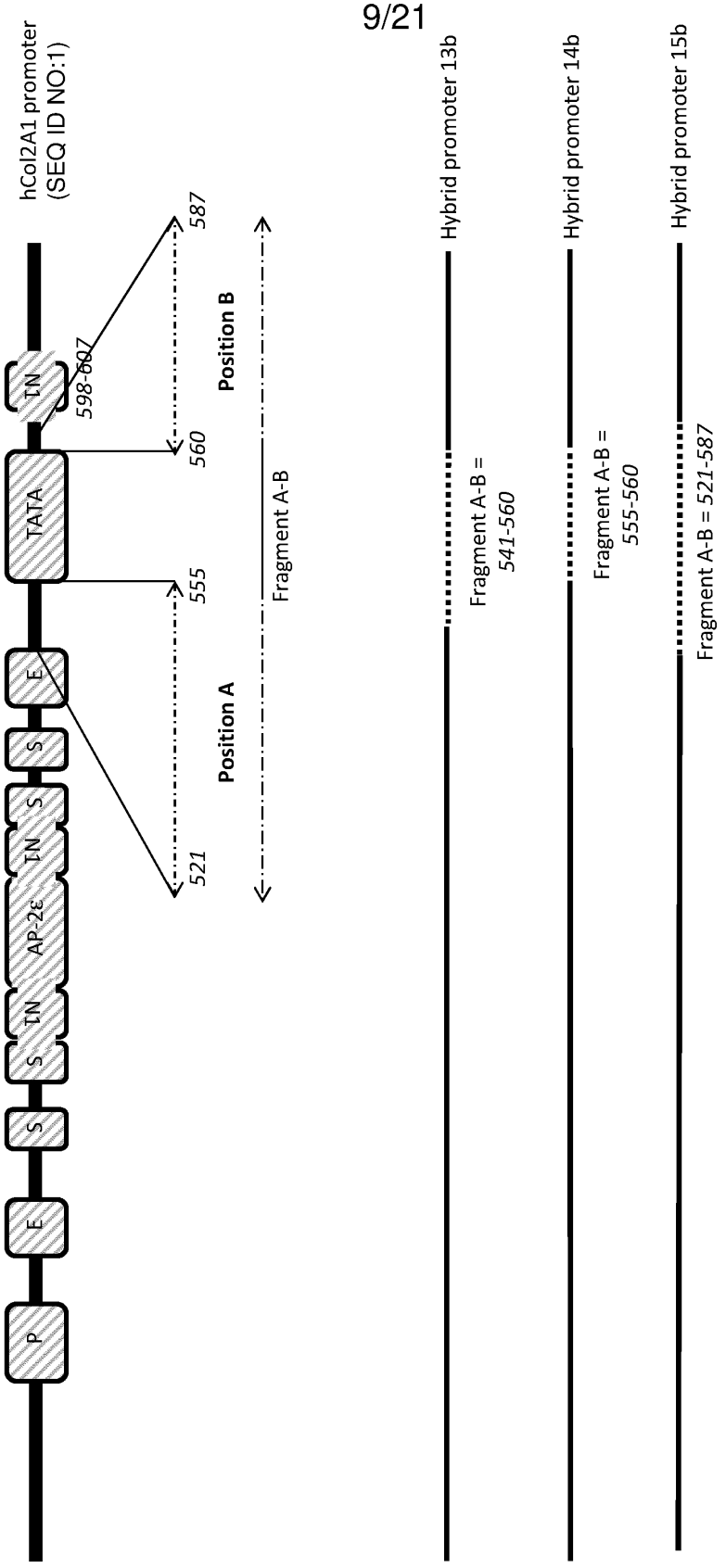

hCol2A1 promoter
(SEQ ID NO:1)
P
E
S
TN
S
AP-2ε
TN
S
S
E
TATA
TN
598-607
587
560
555
521
Position B
Position A
Fragment A-B
Hybrid promoter 13b
Fragment A-B =
541-560
Hybrid promoter 14b
Fragment A-B =
555-560
Hybrid promoter 15b
Fragment A-B = 521-587

FIG.9

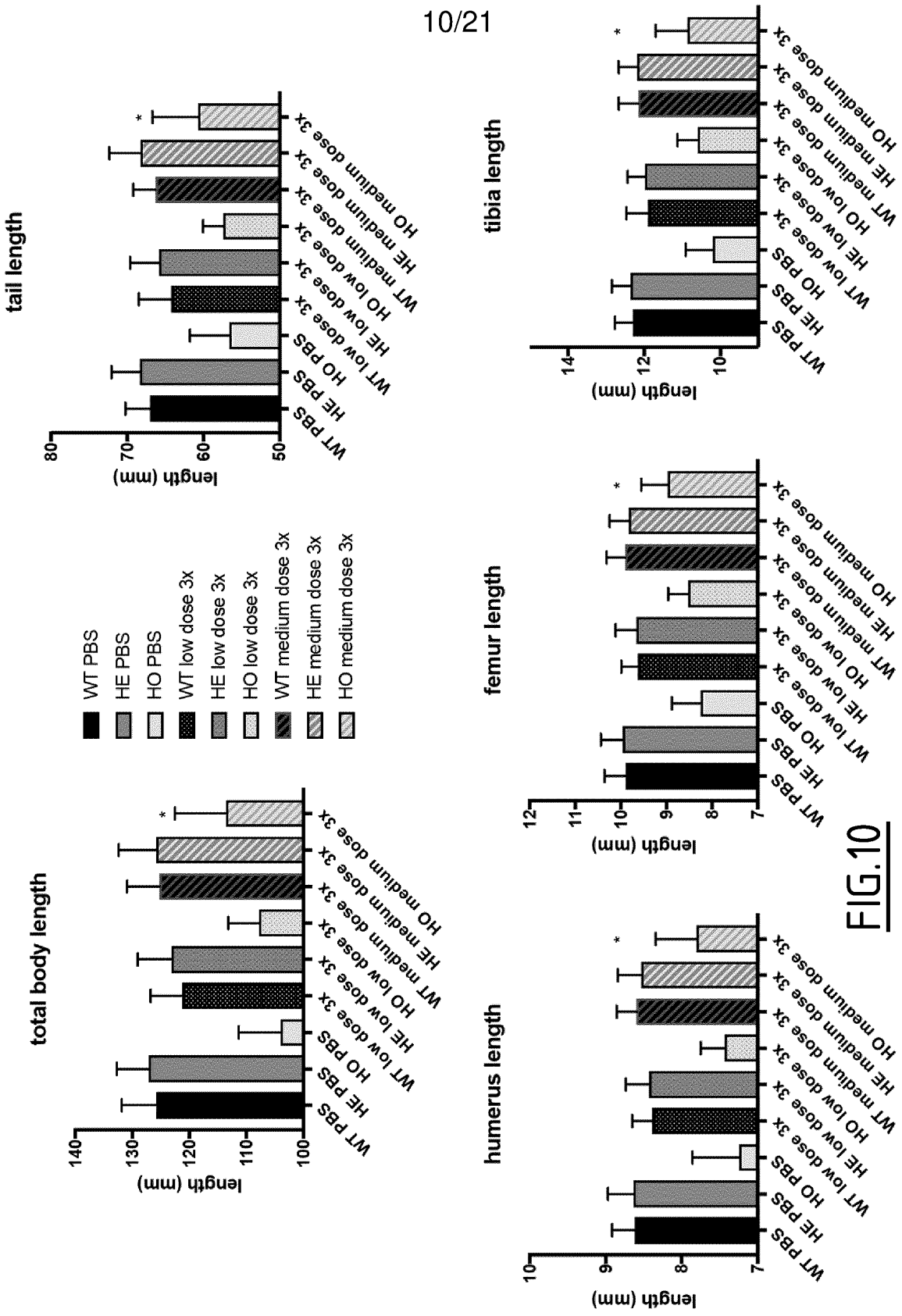

WT PBS
HE PBS
HO PBS
WT low dose 3x
HE low dose 3x
HO low dose 3x
WT medium dose 3x
HE medium dose 3x
HO medium dose 3x
tail length
length (mm)
total body length
length (mm)
tibia length
length (mm)
femur length
length (mm)
humerus length
length (mm)

FIG.10

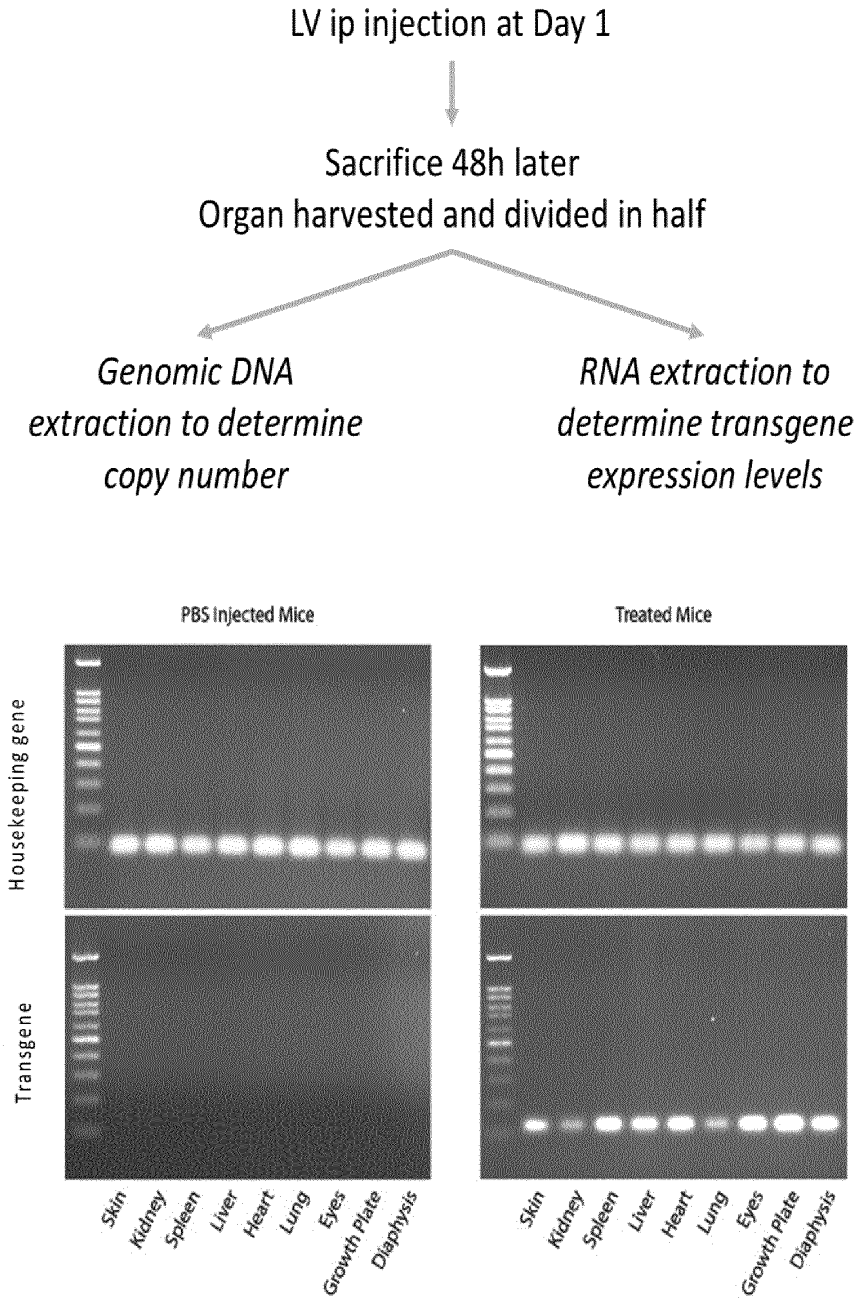

LV ip injection at Day 1
Sacrifice 48h later
Organ harvested and divided in half
Genomic DNA extraction to determine copy number
RNA extraction to determine transgene expression levels
PBS Injected Mice
Treated Mice
Housekeeping gene
Transgene
Skin
Kidney
Spleen
Liver
Heart
Lung
Eyes
Growth Plate
Diaphysis

FIG.11

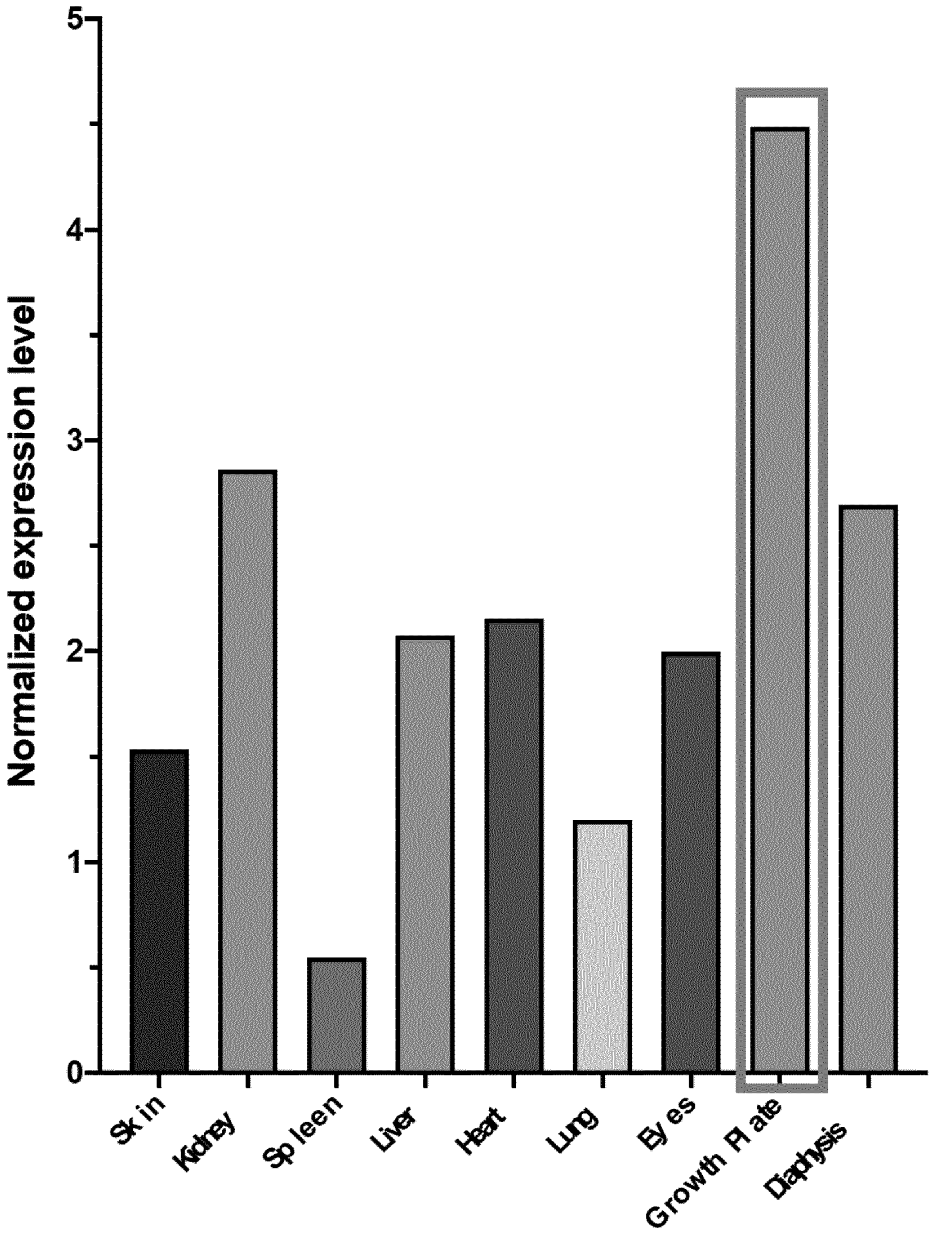

Normalized expression level
5
4
3
2
1
0
Skin
Kidney
Spleen
Liver
Heart
Lung
Eyes
Growth Plate
Diaphysis

FIG.11 end

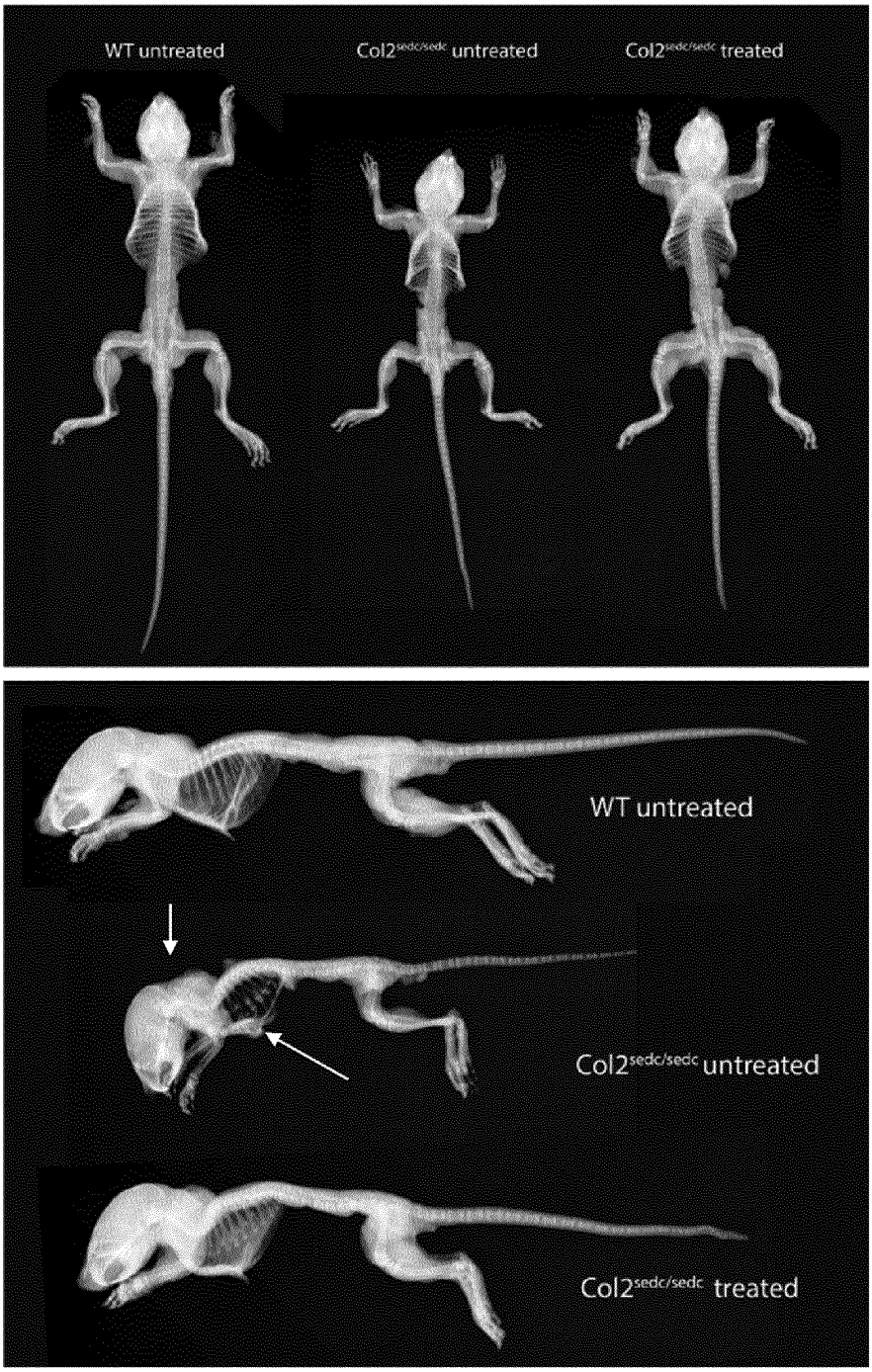
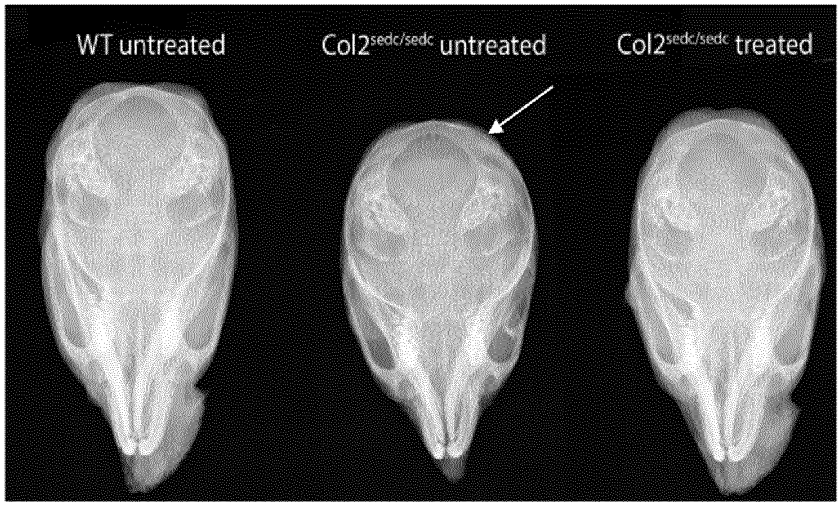

WT untreated
Col2sedc/sedc untreated
Col2sedc/sedc treated
WT untreated
Col2sedc/sedc untreated
Col2sedc/sedc treated

FIG.12



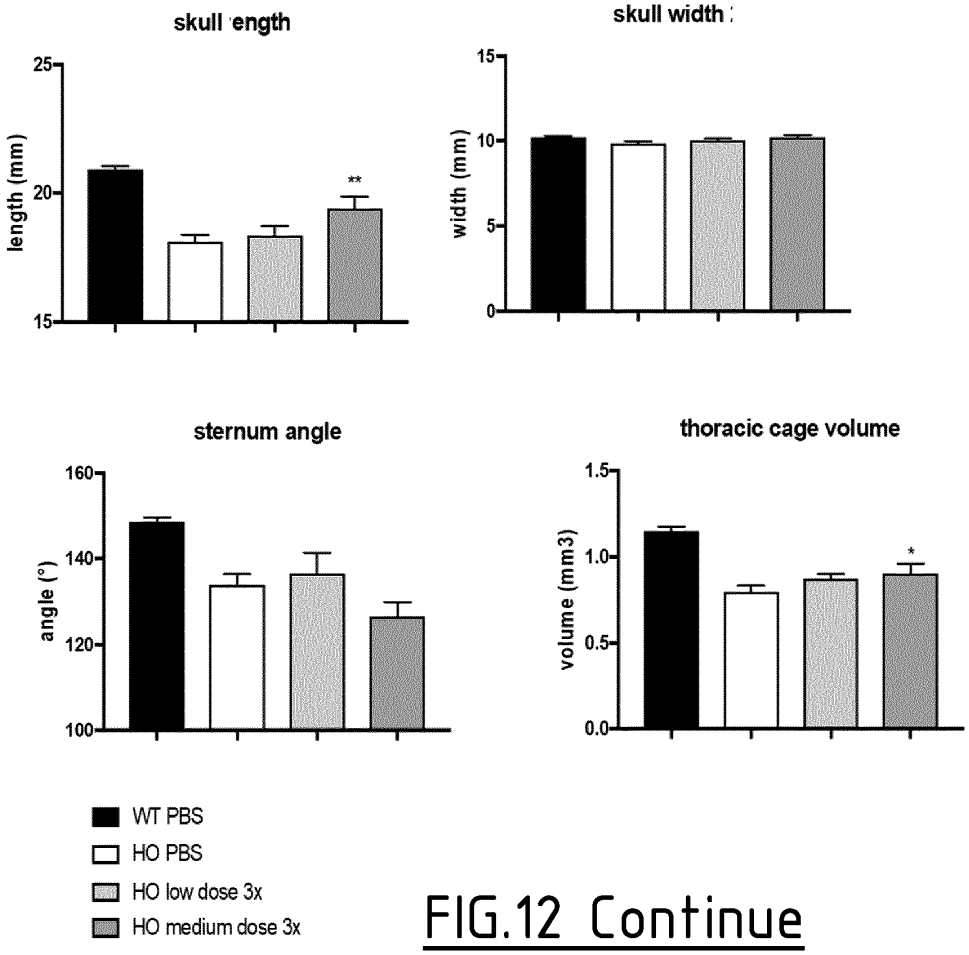


FIG.12 Continue

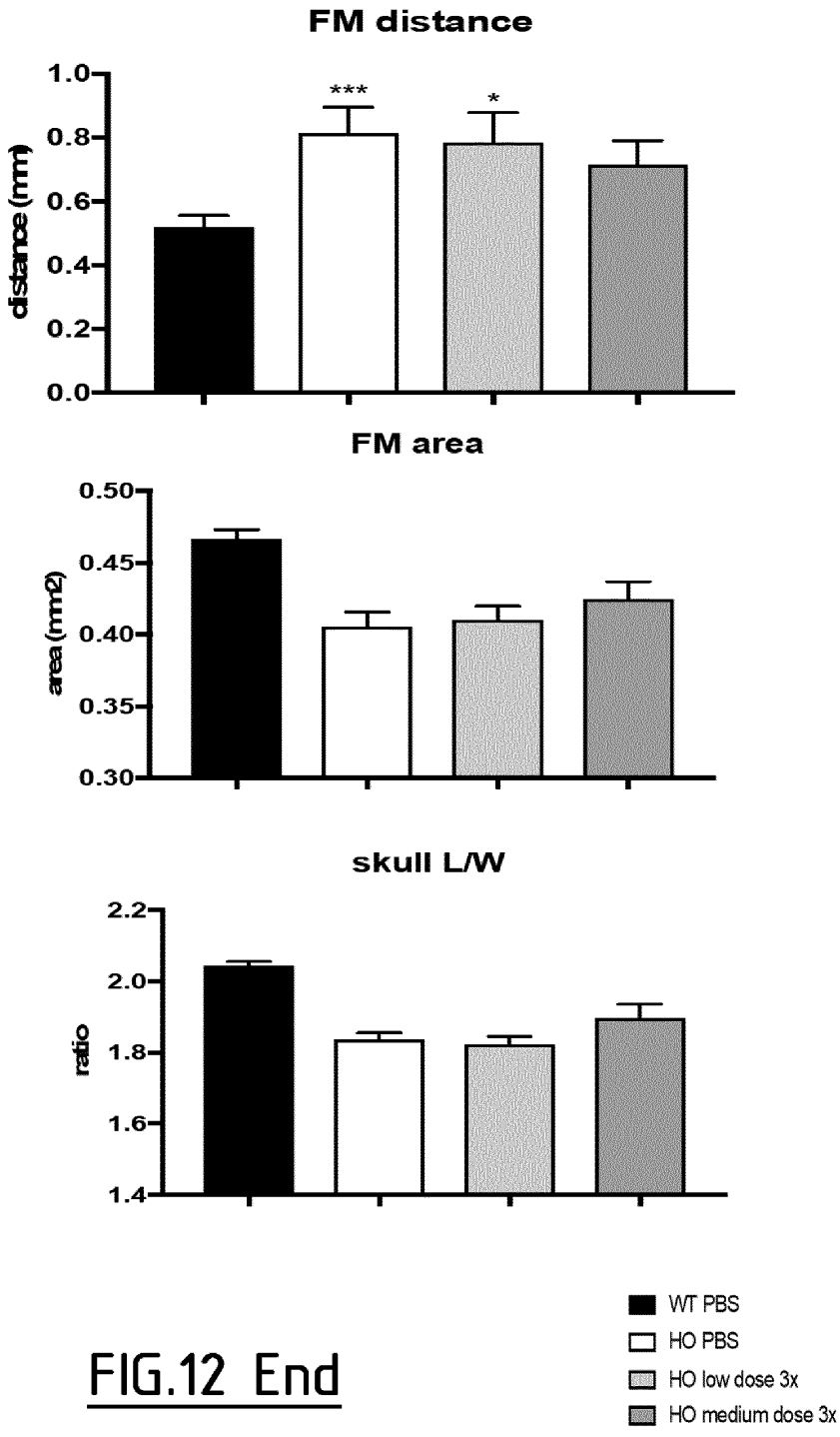
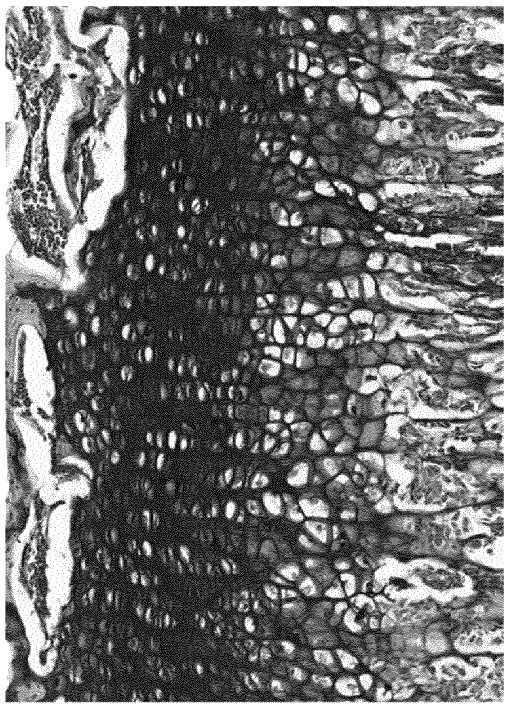
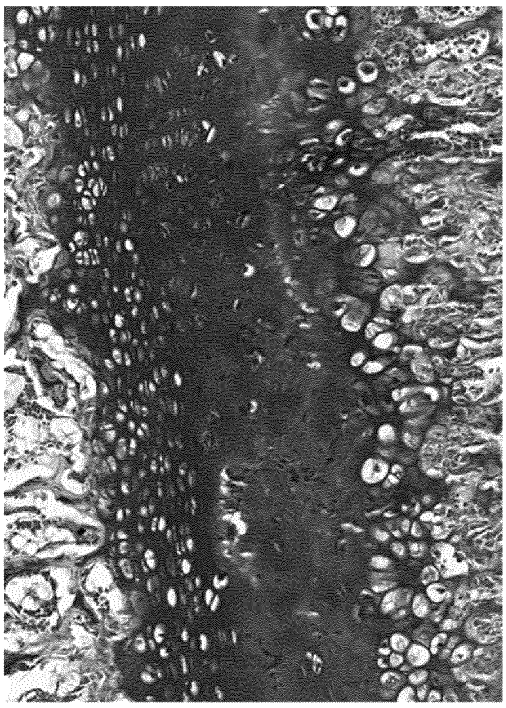

FM distance
distance (mm)
1.0
0.8
0.6
0.4
0.2
0.0
***
*
FM area
area (mm2)
0.50
0.45
0.40
0.35
0.30
skull L/W
ratio
2.2
2.0
1.8
1.6
1.4
WT PBS
HO PBS
HO low dose 3x
HO medium dose 3x

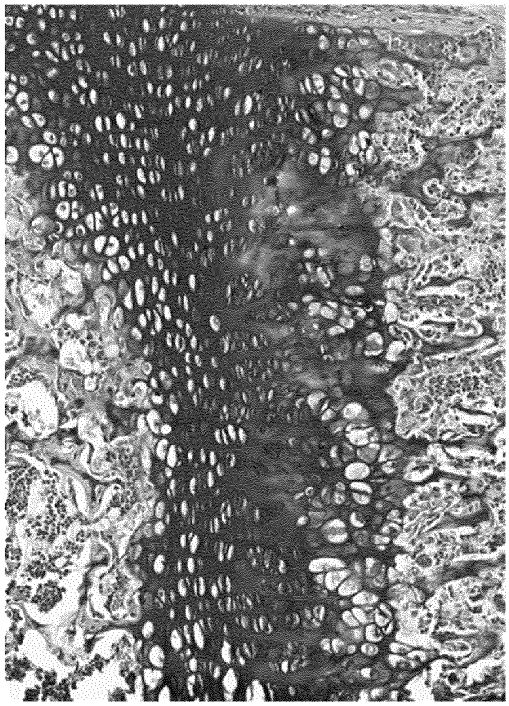
FIG.12 End



WT



HO, PBS



HO, treated

Magnification, 20X

FIG.13

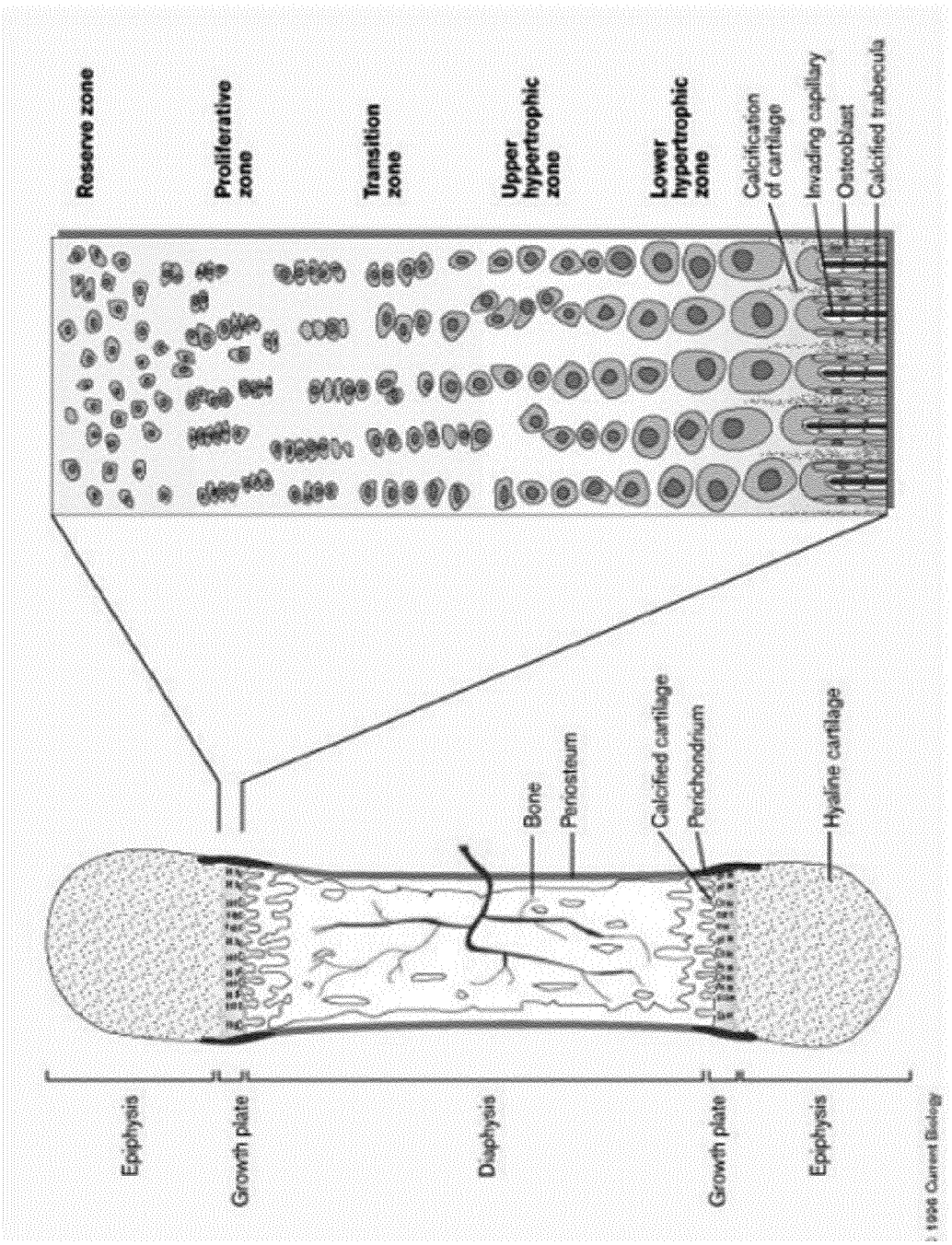

Reserve zone
Proliferative zone
Transition zone
Upper hypertrophic zone
Lower hypertrophic zone
Calcification of cartilage
Invading capillary
Osteoblast
Calcified trabecula
Bone
Periosteum
Calcified cartilage
Perichondrium
Hyaline cartilage
Epiphysis
Growth plate
Diaphysis
Growth plate
Epiphysis

FIG.13 End

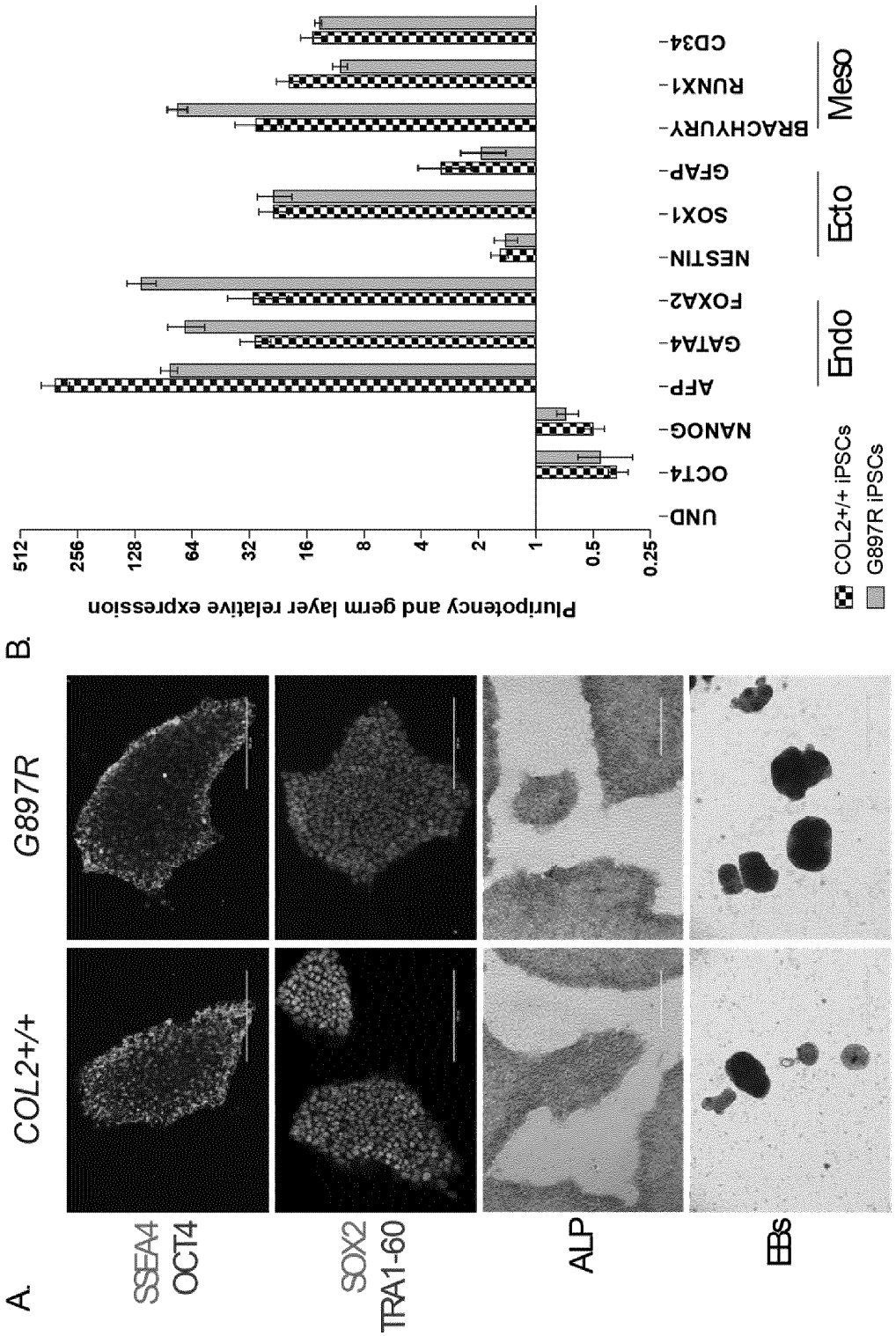

A.
COL2+/+
G897R
SSEA4
OCT4
SOX2
TRA1-60
ALP
EBs
B.
Pluripotency and germ layer relative expression
512
256
128
64
32
16
8
4
2
1
0.5
0.25
UND-
OCT4-
NANOG-
AFP-
GATA4-
FOXA2-
NESTIN-
SOX1-
GFAP-
BRACHYURY-
RUNX1-
CD34-
Endo
Ecto
Meso
COL2+/+ iPSCs
G897R iPSCs

FIG.14

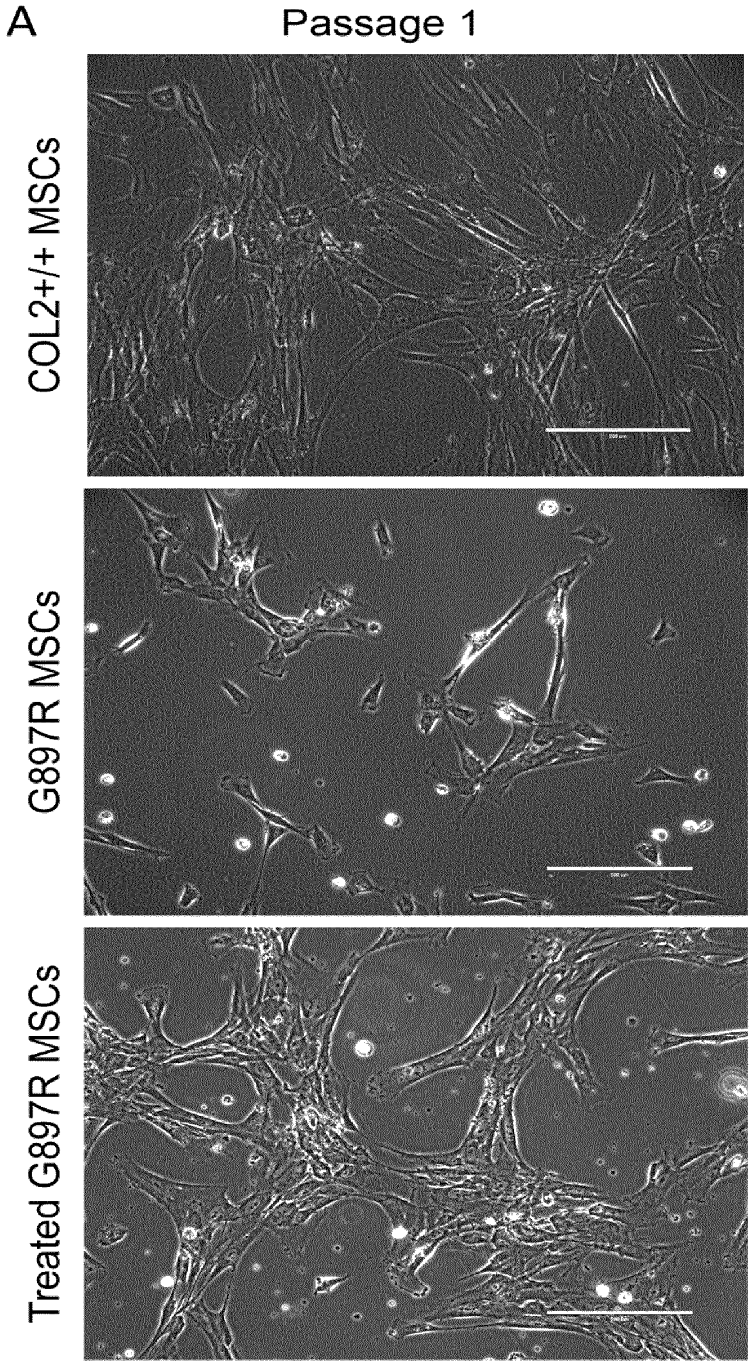


FIG.15

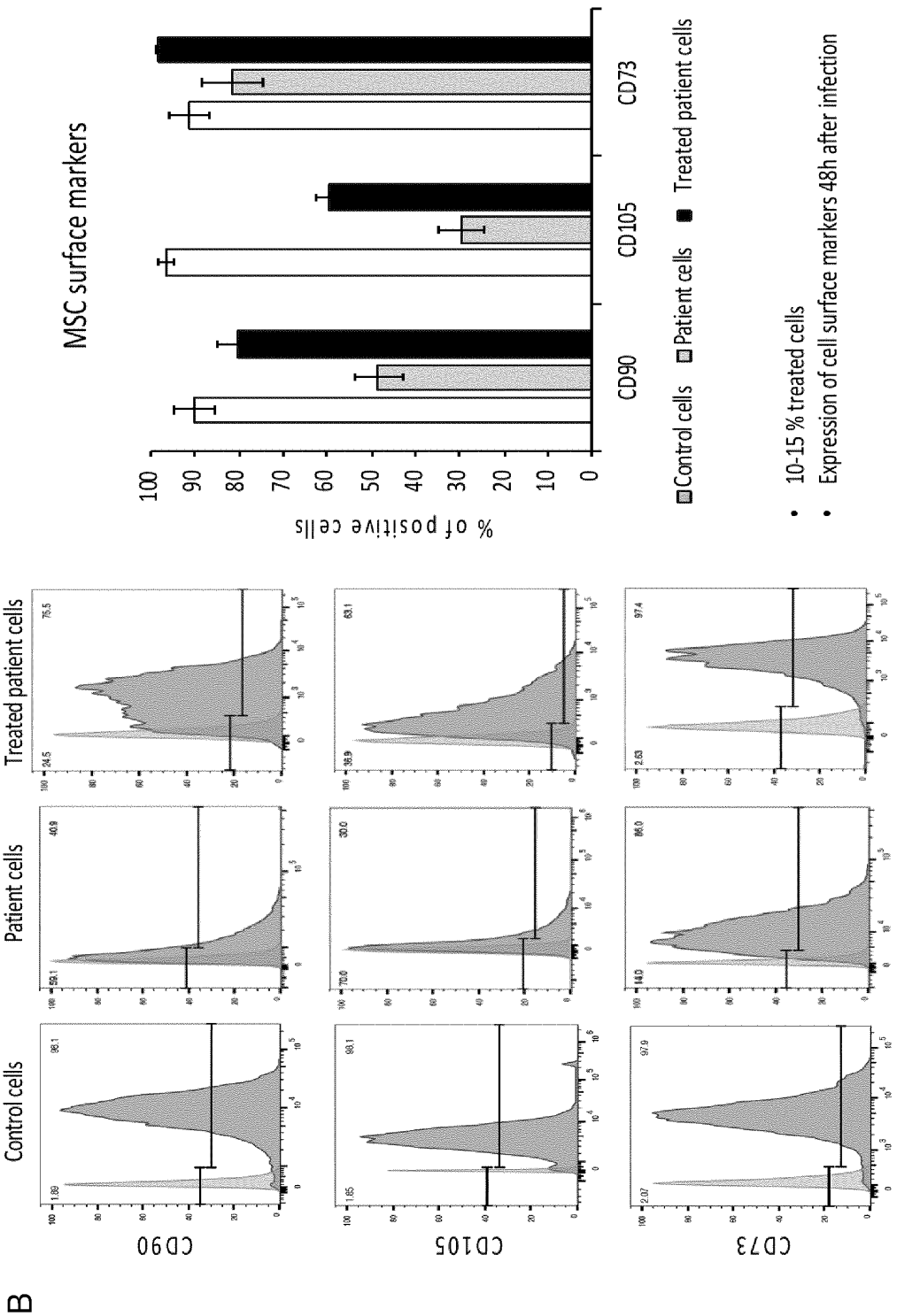

B
Control cells
Patient cells
Treated patient cells
CD90
CD105
CD73
MSC surface markers
% of positive cells
CD90
CD105
CD73
Control cells
Patient cells
Treated patient cells
10-15 % treated cells
Expression of cell surface markers 48h after infection

FIG.15 End

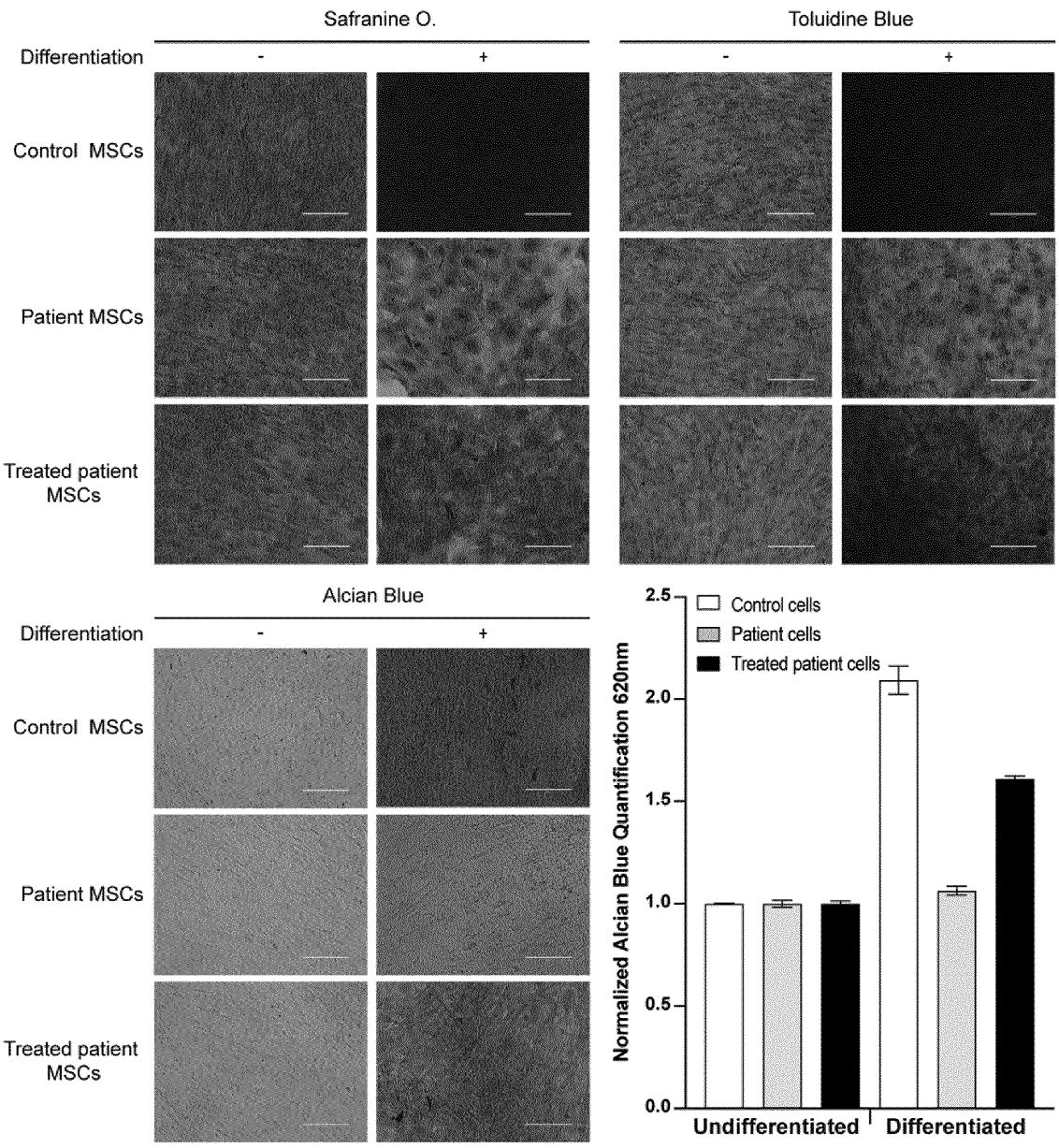

Safranine O.
Toluidine Blue
Differentiation
-
+
Control MSCs
Patient MSCs
Treated patient MSCs
Alcian Blue
Normalized Alcian Blue Quantification 620nm
Control cells
Patient cells
Treated patient cells
2.5
2.0
1.5
1.0
0.5
0.0
Undifferentiated
Differentiated

FIG.16