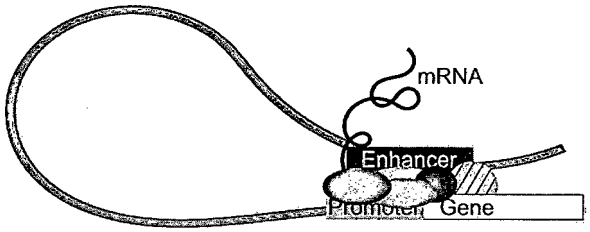
Enhancer/promoter interactions



Enhancer regulatory elements induce gene expression via looping



Inducing a DSB within the enhancer regulatory region prevents loop-mediated contact and abrogates gene expression

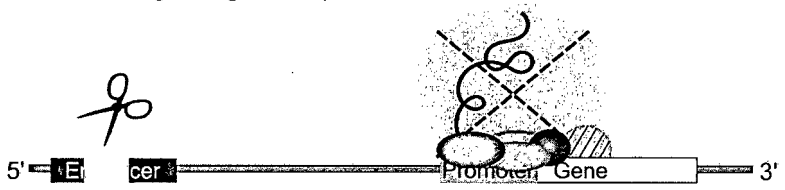


Figure 1

Regulatory sites that determine loop structure

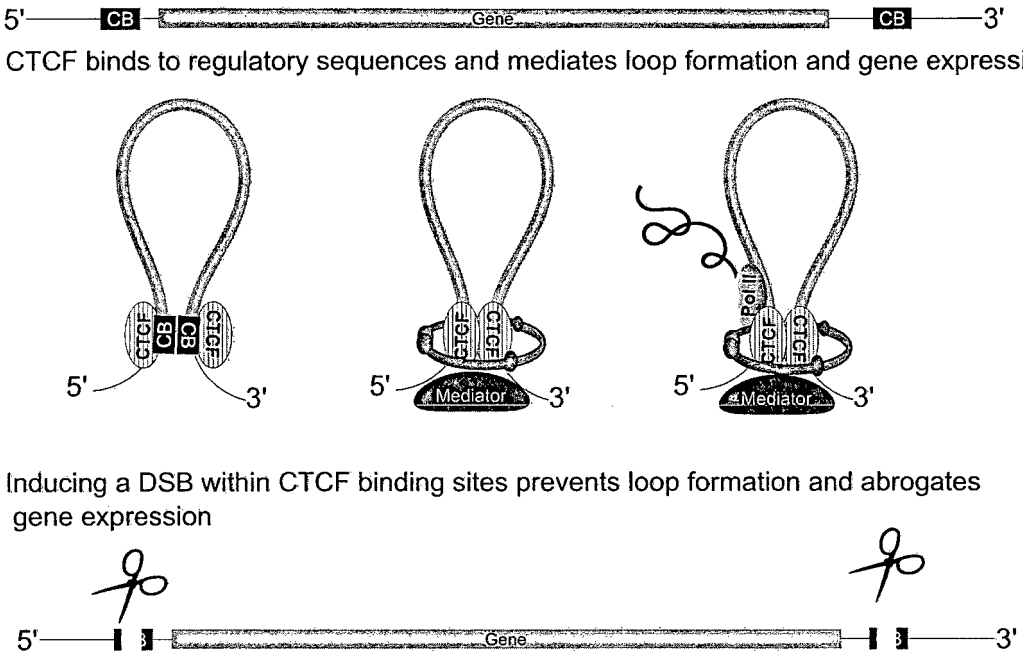


Figure 2

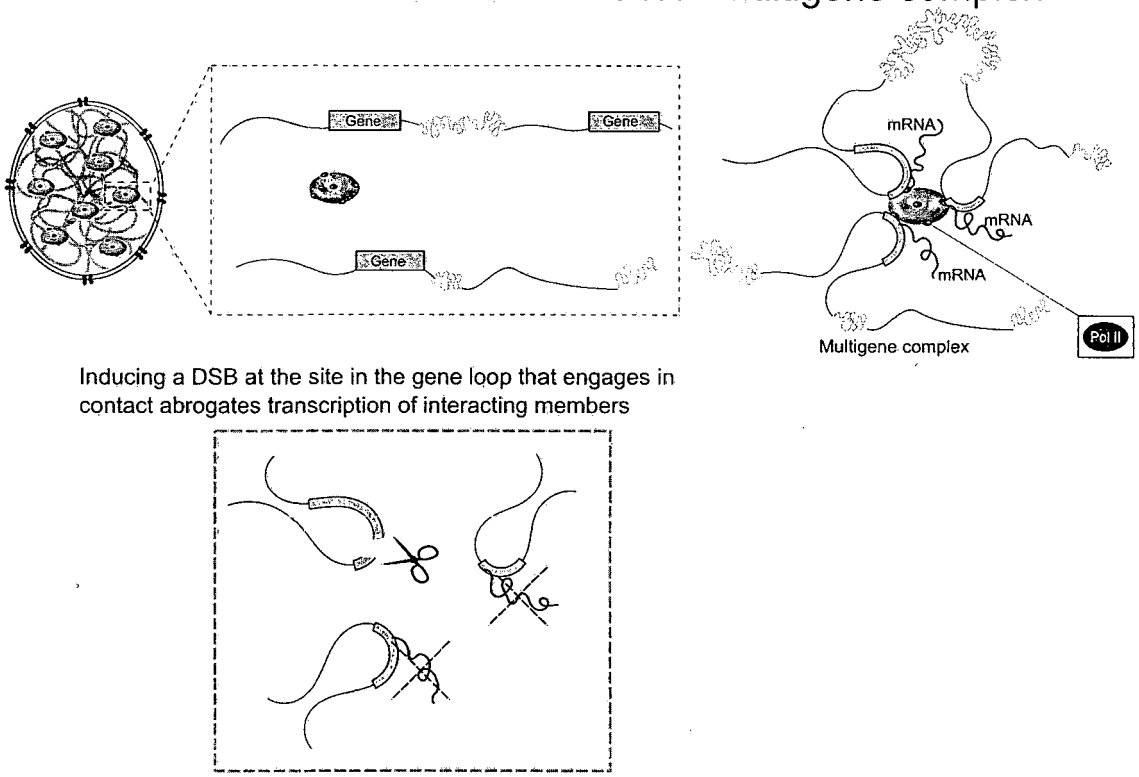


Figure 3

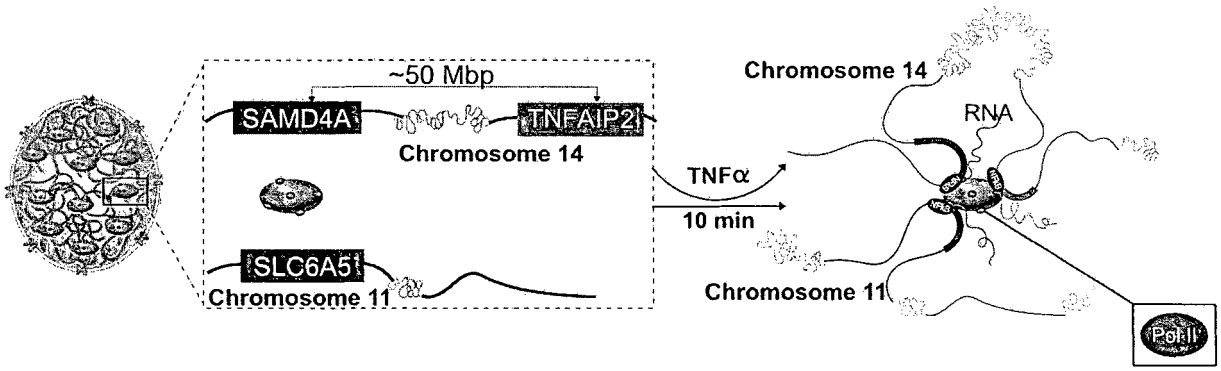
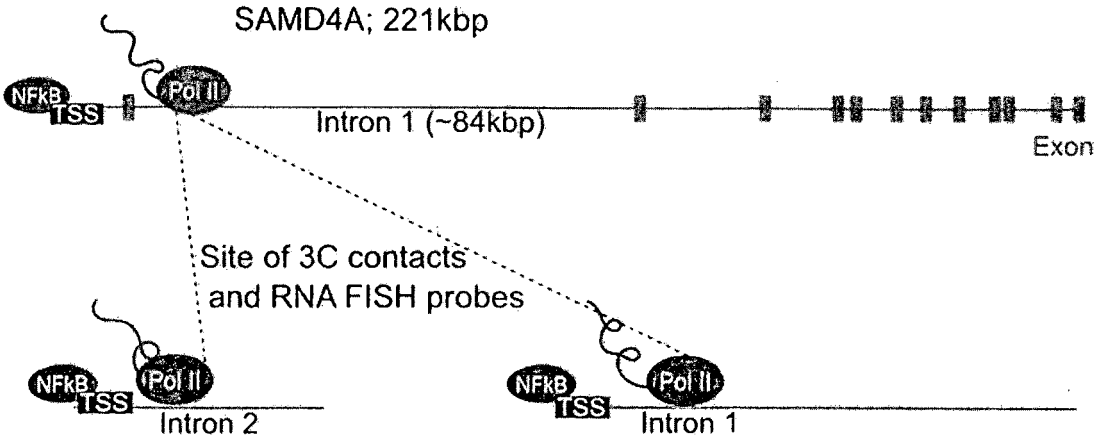
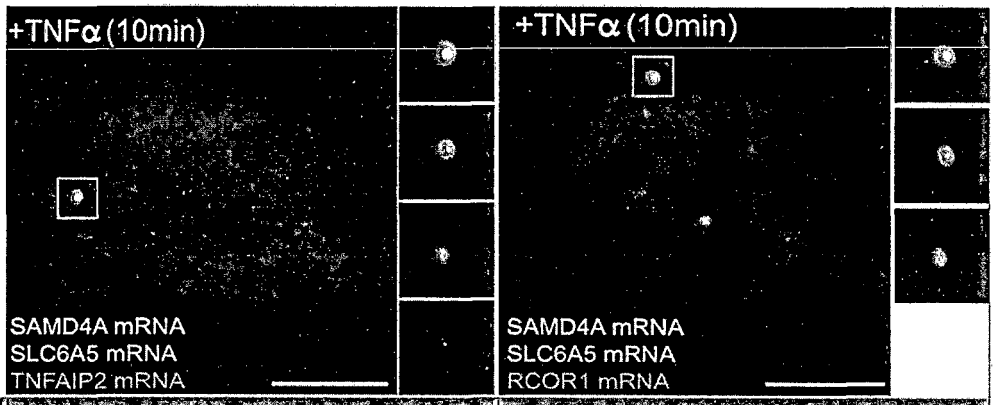


Figure 4





n (%)	SAMD4A/TNFAIP2/SLC6A5	RCOR1/SAMD4A/SLC6A5
No co-localization	315 (94.9%)	212 (100%)
Triplet co-localization	17 (5.1%)***	0 (0%)

Figure 5

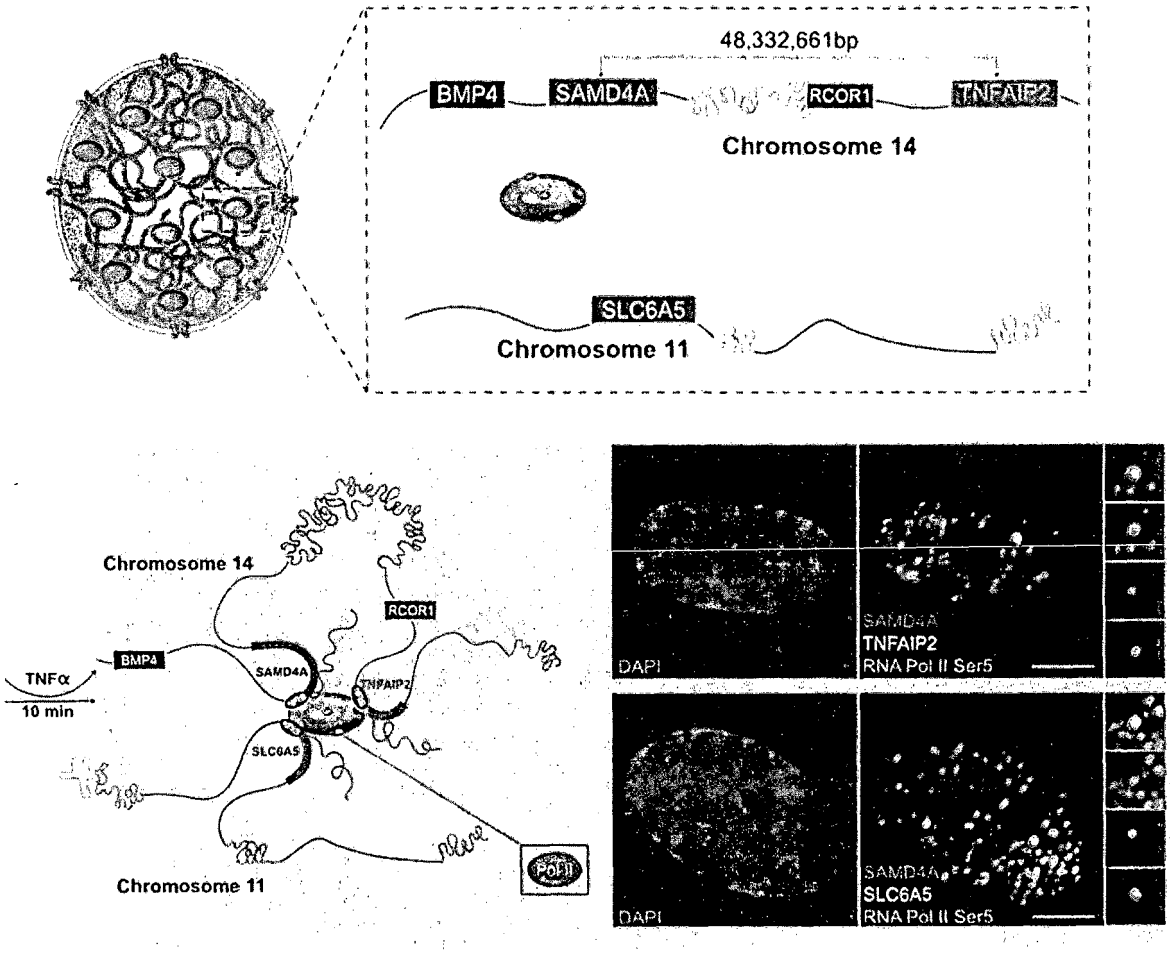

48,332,661bp
BMP4
SAMD4A
RCOR1
TNFAIP2
Chromosome 14
SLC6A5
Chromosome 11
Chromosome 14
RCOR1
TNFα
10 min
BMP4
SAMD4A
TNFAIP2
SLC6A5
Pol II
Chromosome 11
DAPI
SAMD4A
TNFAIP2
RNA Pol II Ser5
DAPI
SAMD4A
SLC6A5
RNA Pol II Ser5

Figure 6

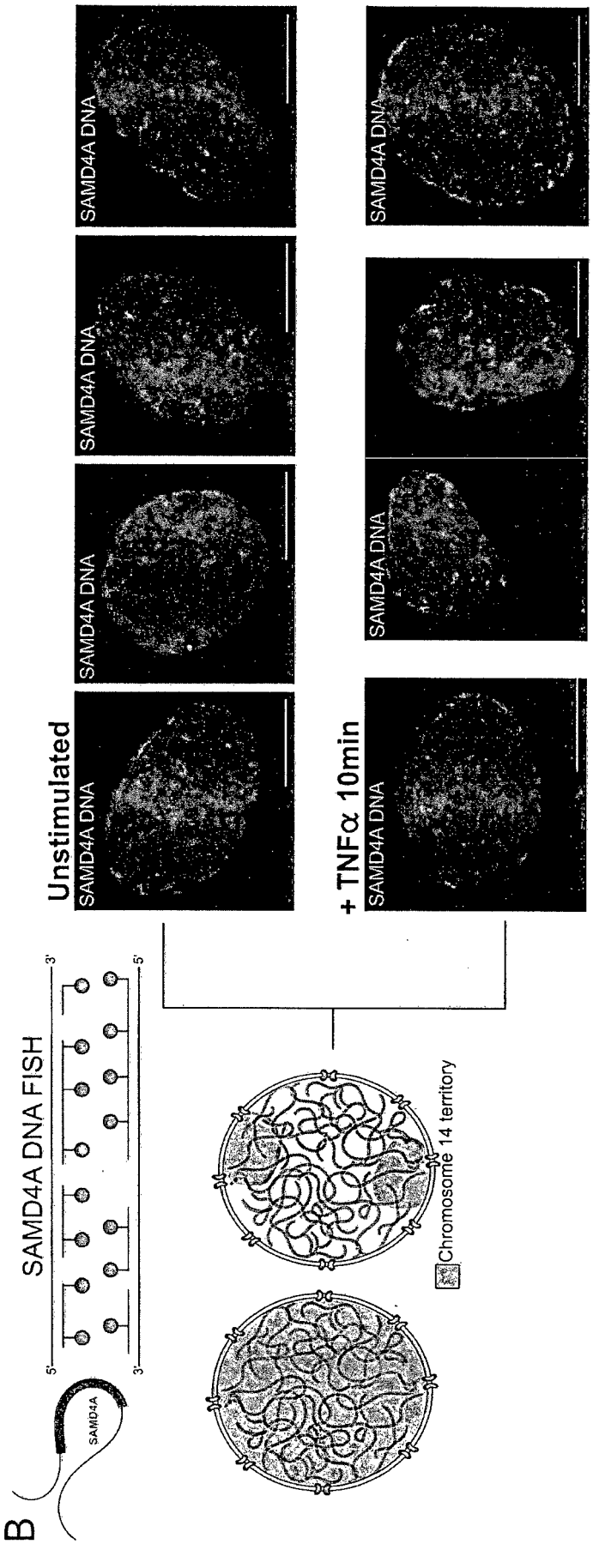

B
SAMD4A DNA FISH
5'
3'
3'
5'
SAMD4A
Chromosome 14 territory
Unstimulated
+ TNFα 10min
SAMD4A DNA

Figure 7

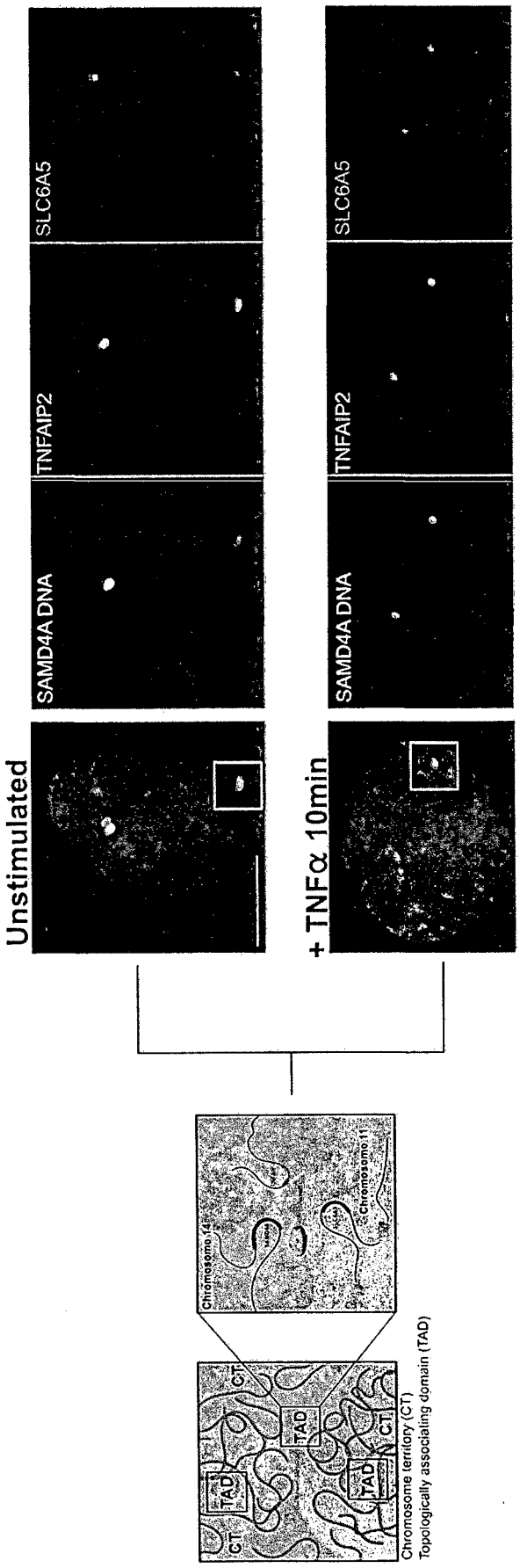

Unstimulated
+ TNFα 10min
SAMD4A DNA
TNFAIP2
SLC6A5
Chromosome territory (CT)
Topologically associating domain (TAD)
CT
TAD

Figure 8

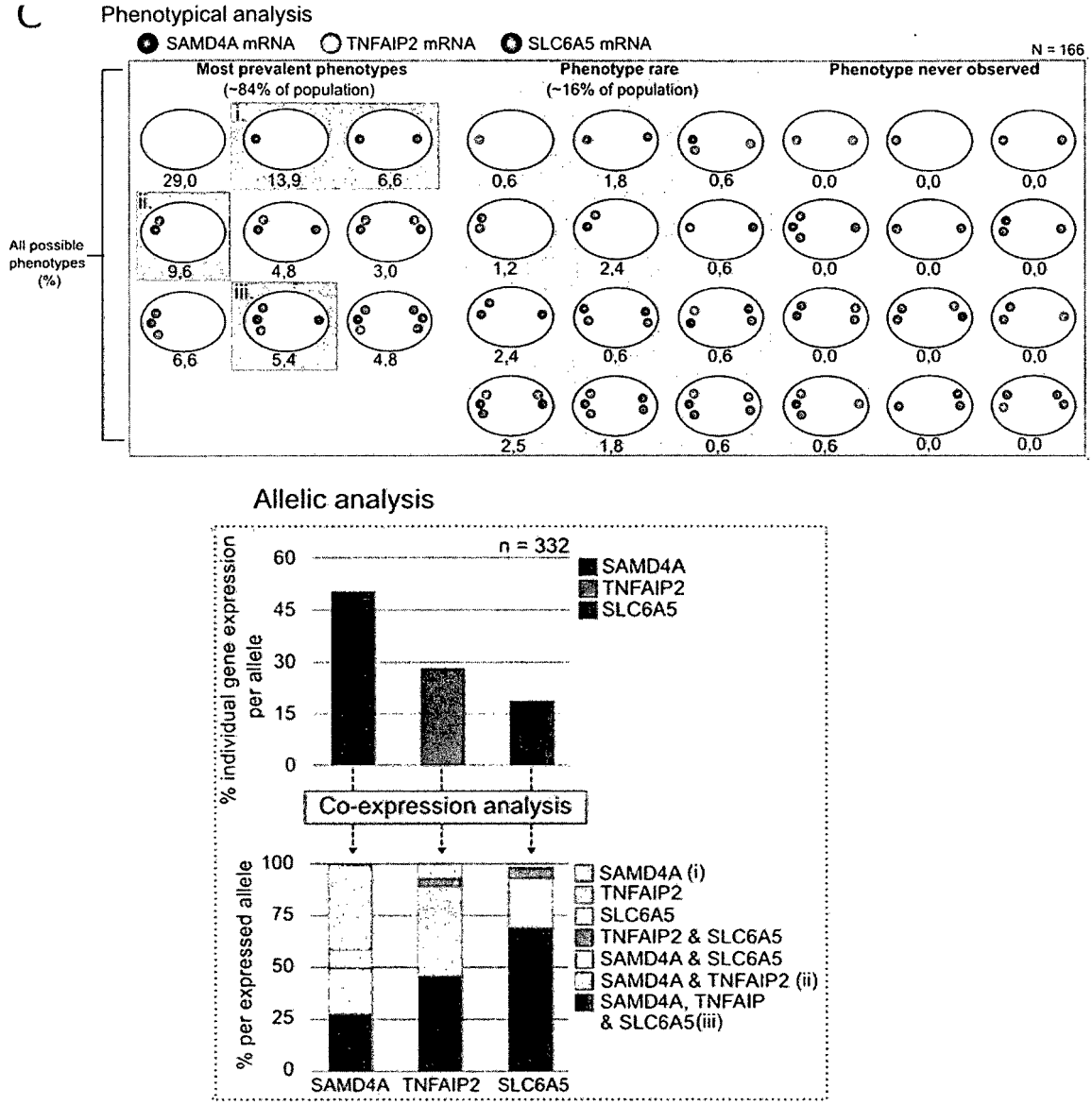

C
Phenotypical analysis
SAMD4A mRNA
TNFAIP2 mRNA
SLC6A5 mRNA
N = 166
Most prevalent phenotypes
(~84% of population)
Phenotype rare
(~16% of population)
Phenotype never observed
All possible phenotypes (%)
i.
ii.
iii.
29,0
13,9
6,6
0,6
1,8
0,6
0,0
0,0
0,0
9,6
4,8
3,0
1,2
2,4
0,6
0,0
0,0
0,0
6,6
5,4
4,8
2,4
0,6
0,6
0,0
0,0
0,0
2,5
1,8
0,6
0,6
0,0
0,0
Allelic analysis
n = 332
% individual gene expression per allele
60
45
30
15
0
SAMD4A
TNFAIP2
SLC6A5
Co-expression analysis
% per expressed allele
100
75
50
25
0
SAMD4A (i)
TNFAIP2
SLC6A5
TNFAIP2 & SLC6A5
SAMD4A & SLC6A5
SAMD4A & TNFAIP2 (ii)
SAMD4A, TNFAIP & SLC6A5(iii)
SAMD4A
TNFAIP2
SLC6A5
i
+TNFα (10min)
SAMD4A mRNA
TNFAIP2 mRNA
SLC6A5 mRNA
SAMD4A
TNFAIP2
SLC6A5
ii
+TNFα (10min)
SAMD4A mRNA
TNFAIP2 mRNA
SLC6A5 mRNA
SAMD4A
TNFAIP2
SLC6A5
iii
+TNFα (10min)
SAMD4A mRNA
TNFAIP2 mRNA
SLC6A5 mRNA
SAMD4A
TNFAIP2
SLC6A5

Figure 9

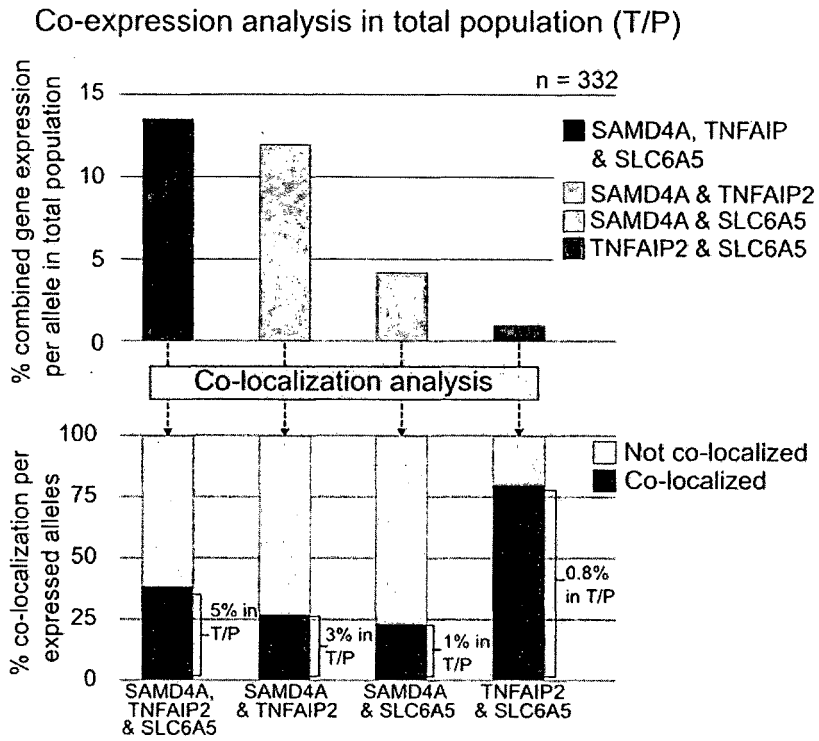


Figure 10

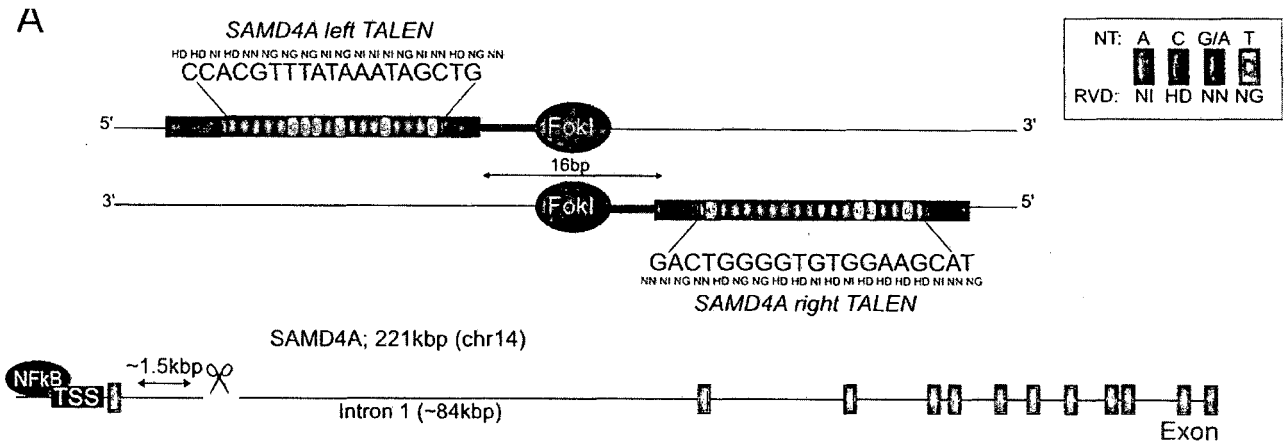


Figure 11

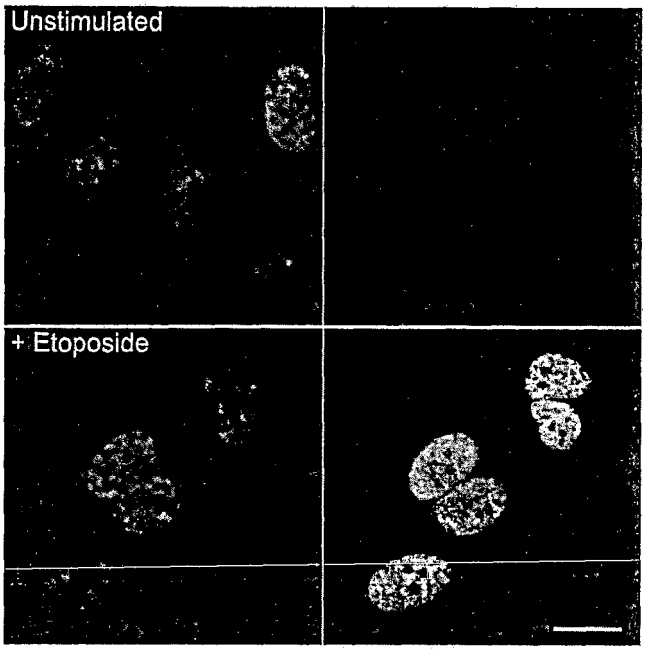


Figure 12

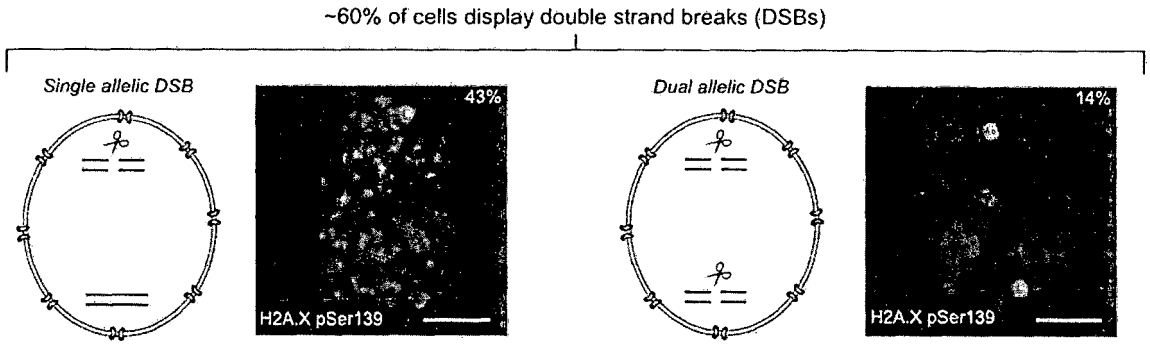


Figure 13

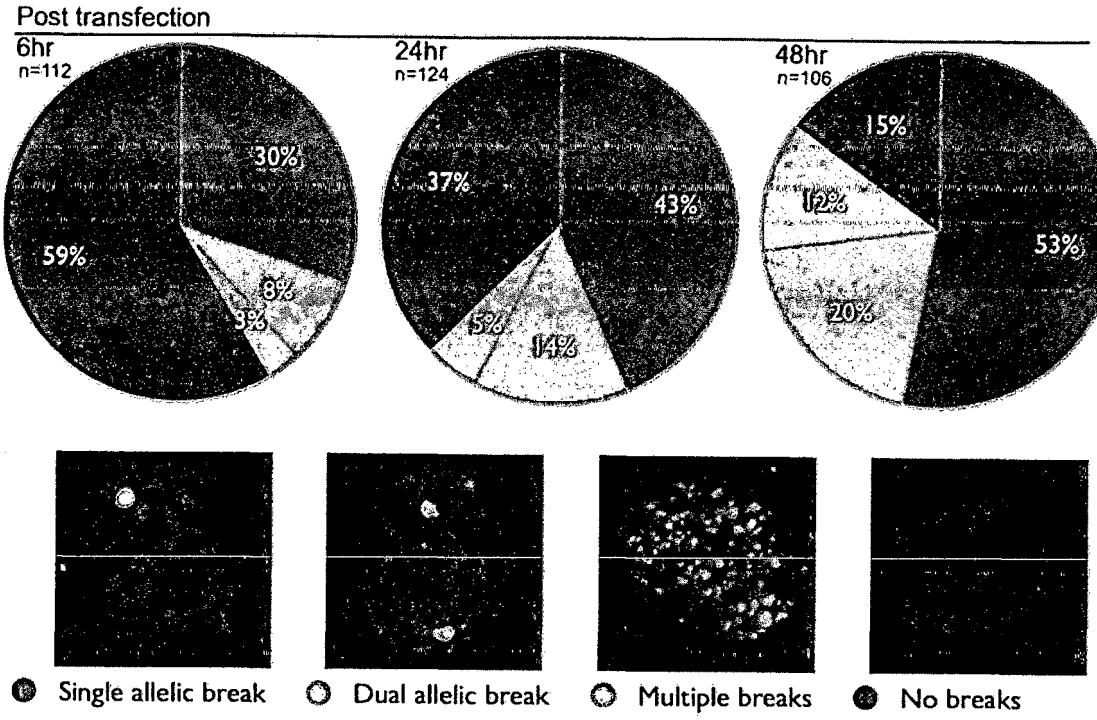


Figure 14

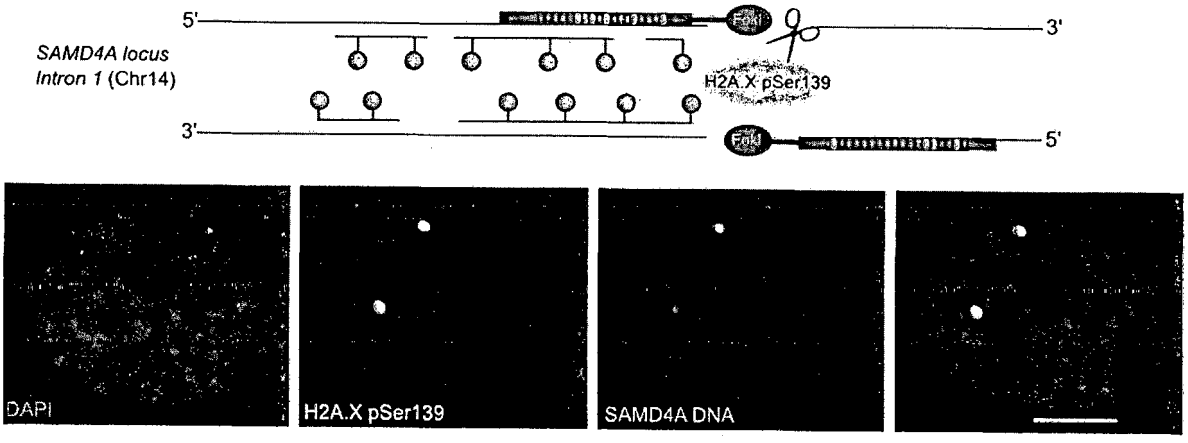


Figure 15

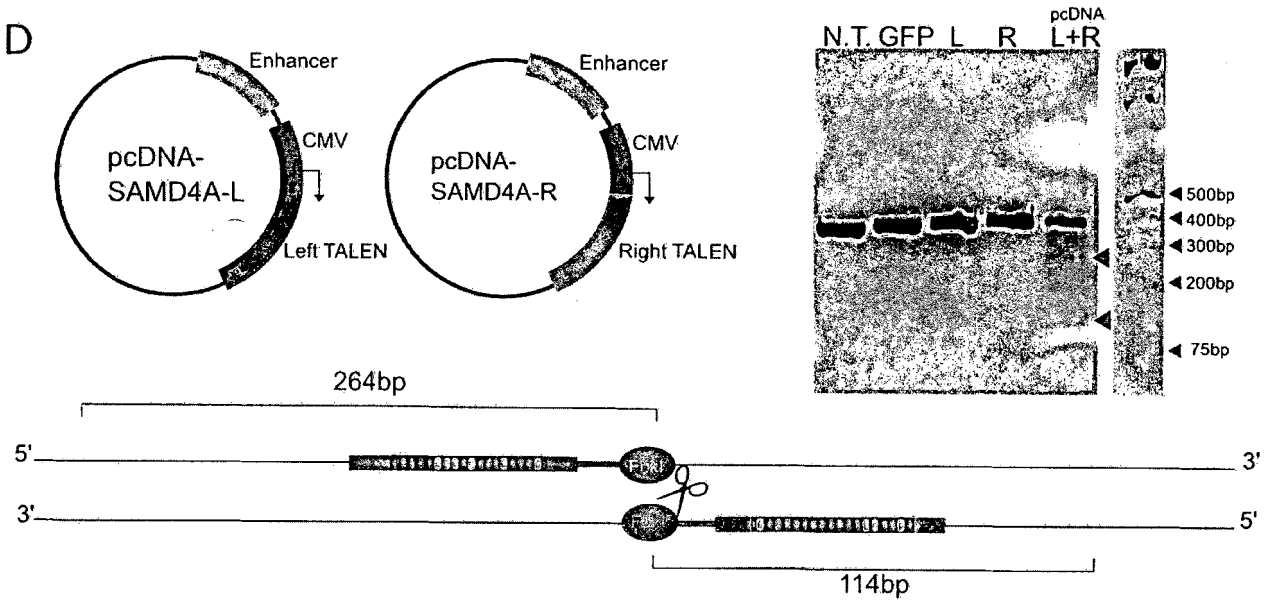


Figure 16

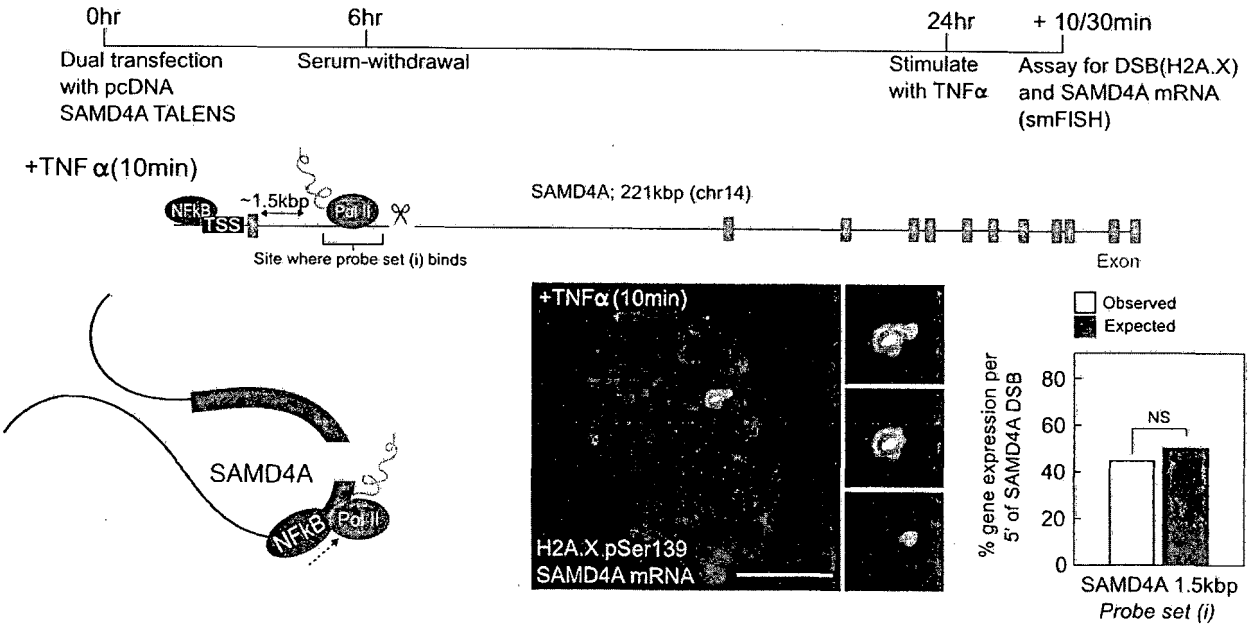


Figure 17

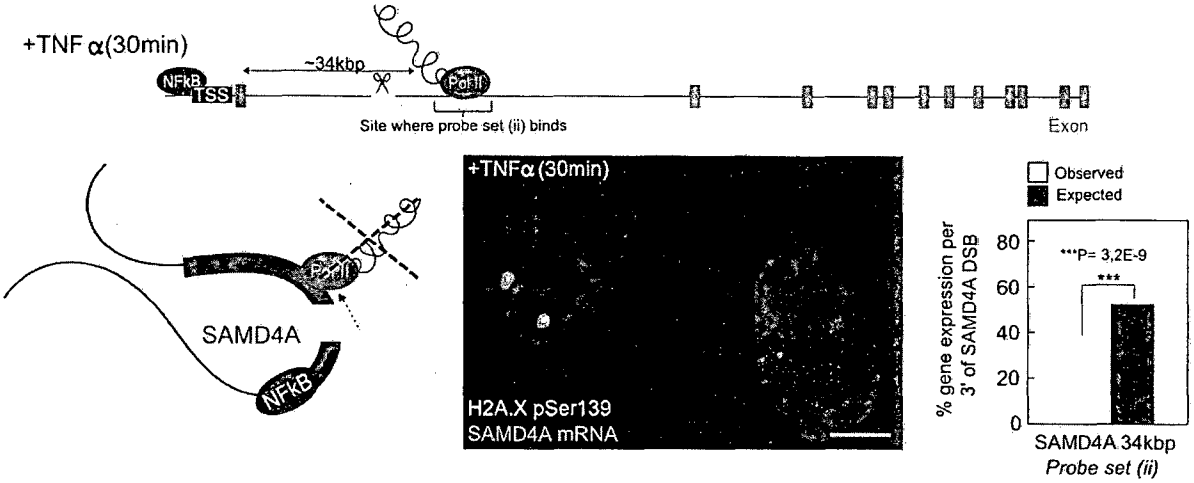


Figure 18

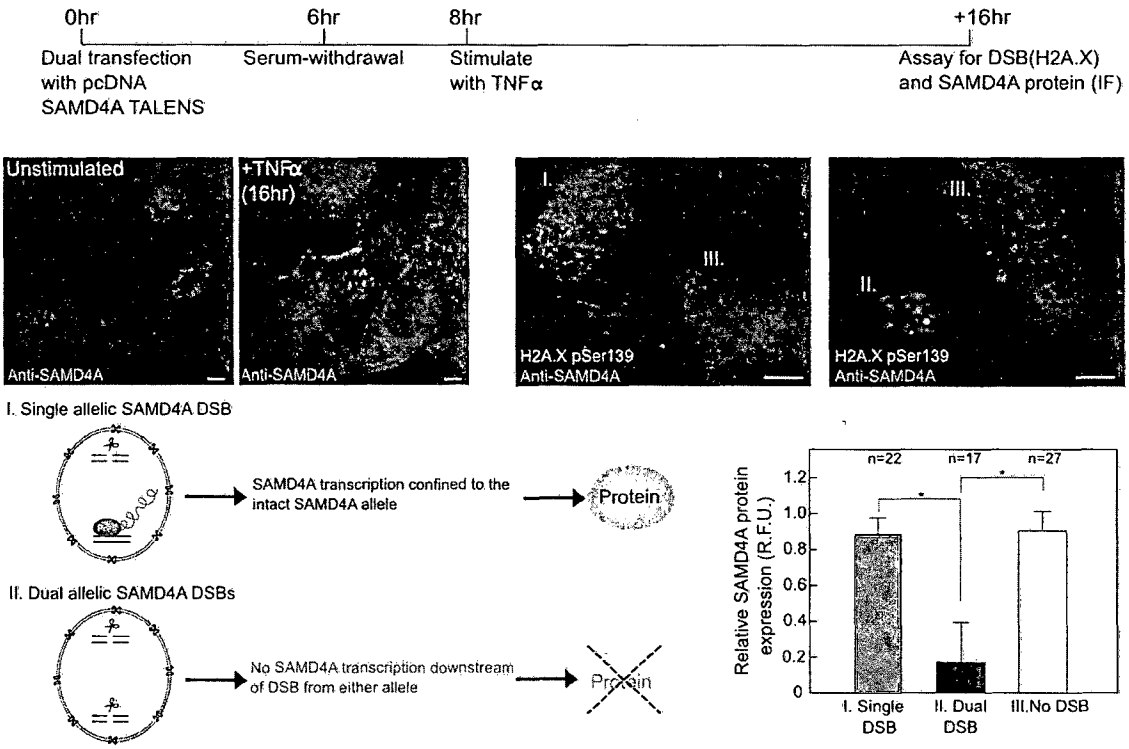


Figure 19

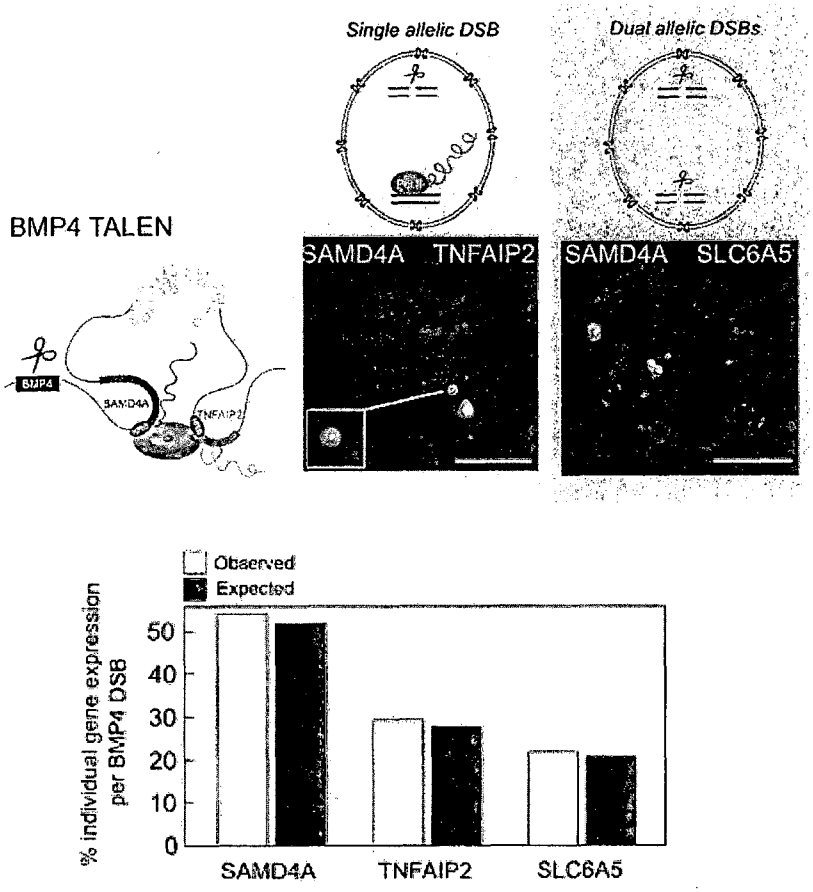

Single allelic DSB
Dual allelic DSBs
BMP4 TALEN
BMP4
SAMD4A
TNFAIP2
SAMD4A TNFAIP2
SAMD4A SLC6A5
Observed
Expected
% individual gene expression per BMP4 DSB
50
40
30
20
10
0
SAMD4A
TNFAIP2
SLC6A5

Figure 20

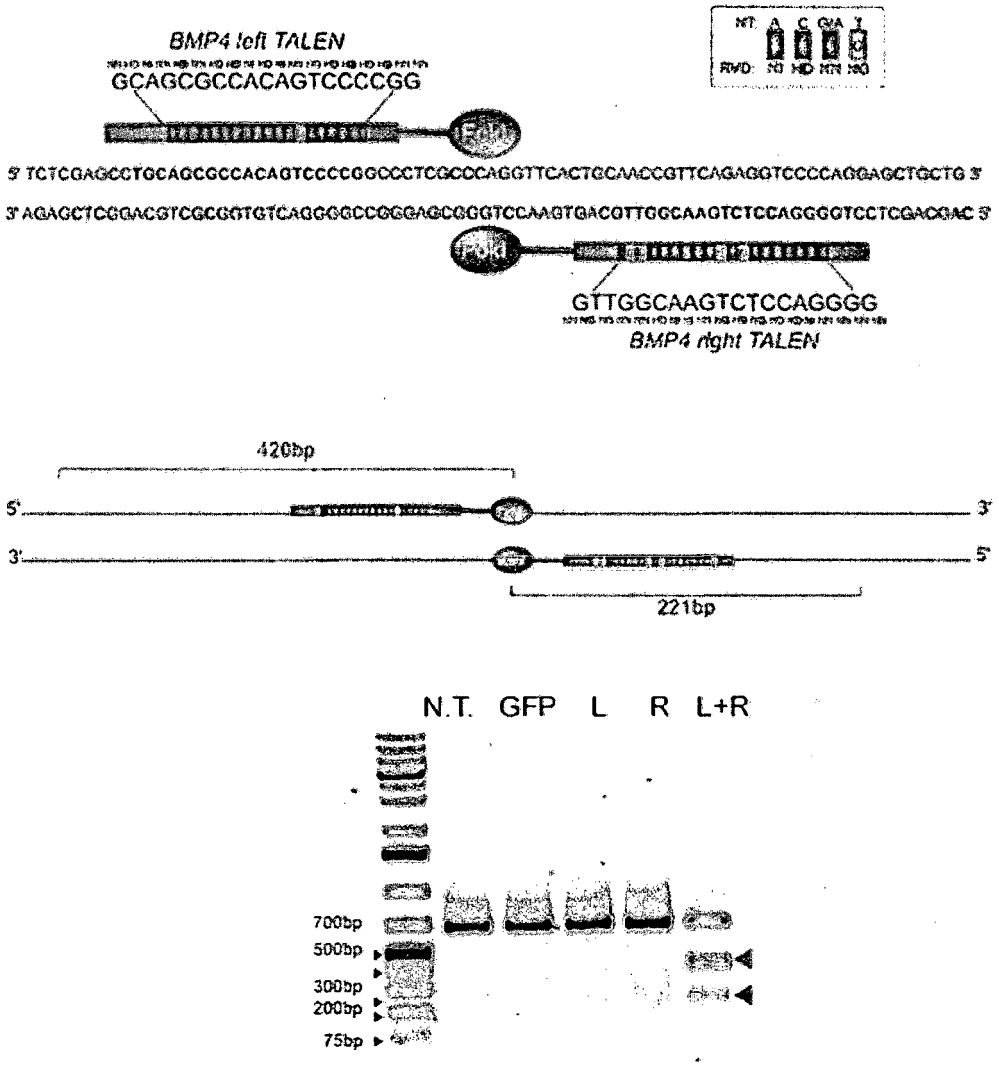

BMP4 left TALEN
GCAGCGCCACAGTCCCCGG
5' TCTCGAGCCTGCAGCGCCACAGTCCCCGGCCCTCGCCCAGGTTCACTGCAACCGTTCAGAGGTCCCCAGGAGCTGCTG 3'
3' AGAGCTCGGACGTCGCGGTGTCAGGGGCCGGGAGCGGGTCCAAGTGACGTTGGCAAGTCTCCAGGGGTCCTCGACGAC 5'
GTTGGCAAGTCTCCAGGGG
BMP4 right TALEN
420bp
5'
3'
3'
5'
221bp
N.T. GFP L R L+R
700bp
500bp
300bp
200bp
75bp

Figure 21

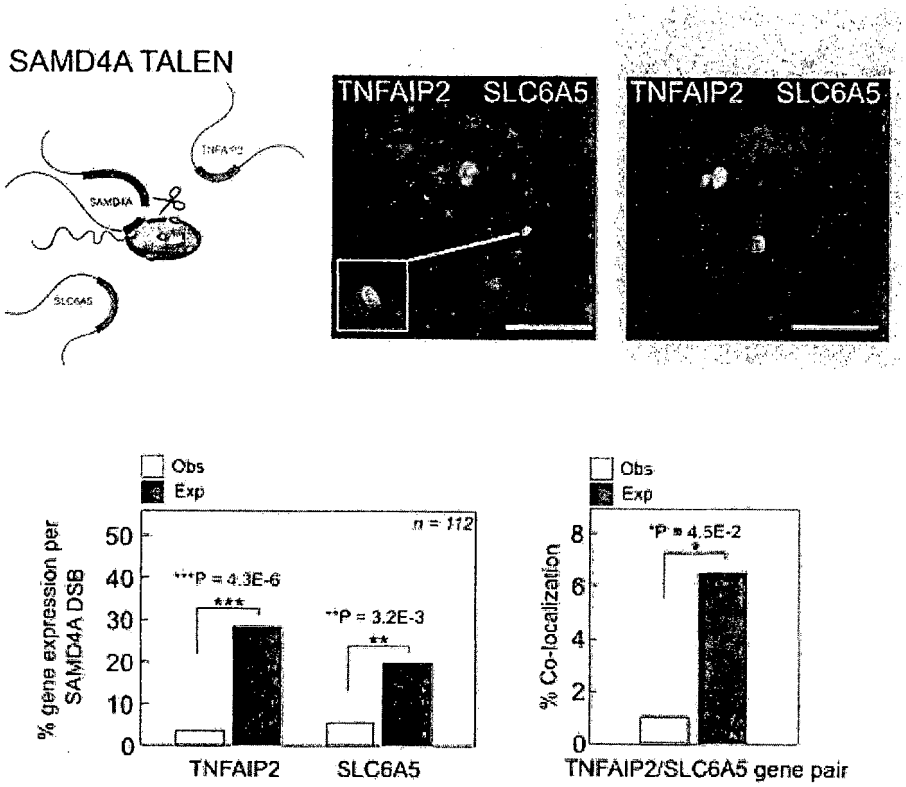

SAMD4A TALEN
TNFAIP2
SAMD4A
SLC6A5
TNFAIP2 SLC6A5
TNFAIP2 SLC6A5
Obs
Exp
% gene expression per SAMD4A DSB
n = 112
***P = 4.3E-6
**P = 3.2E-3
TNFAIP2
SLC6A5
Obs
Exp
% Co-localization
*P = 4.5E-2
TNFAIP2/SLC6A5 gene pair

Figure 22

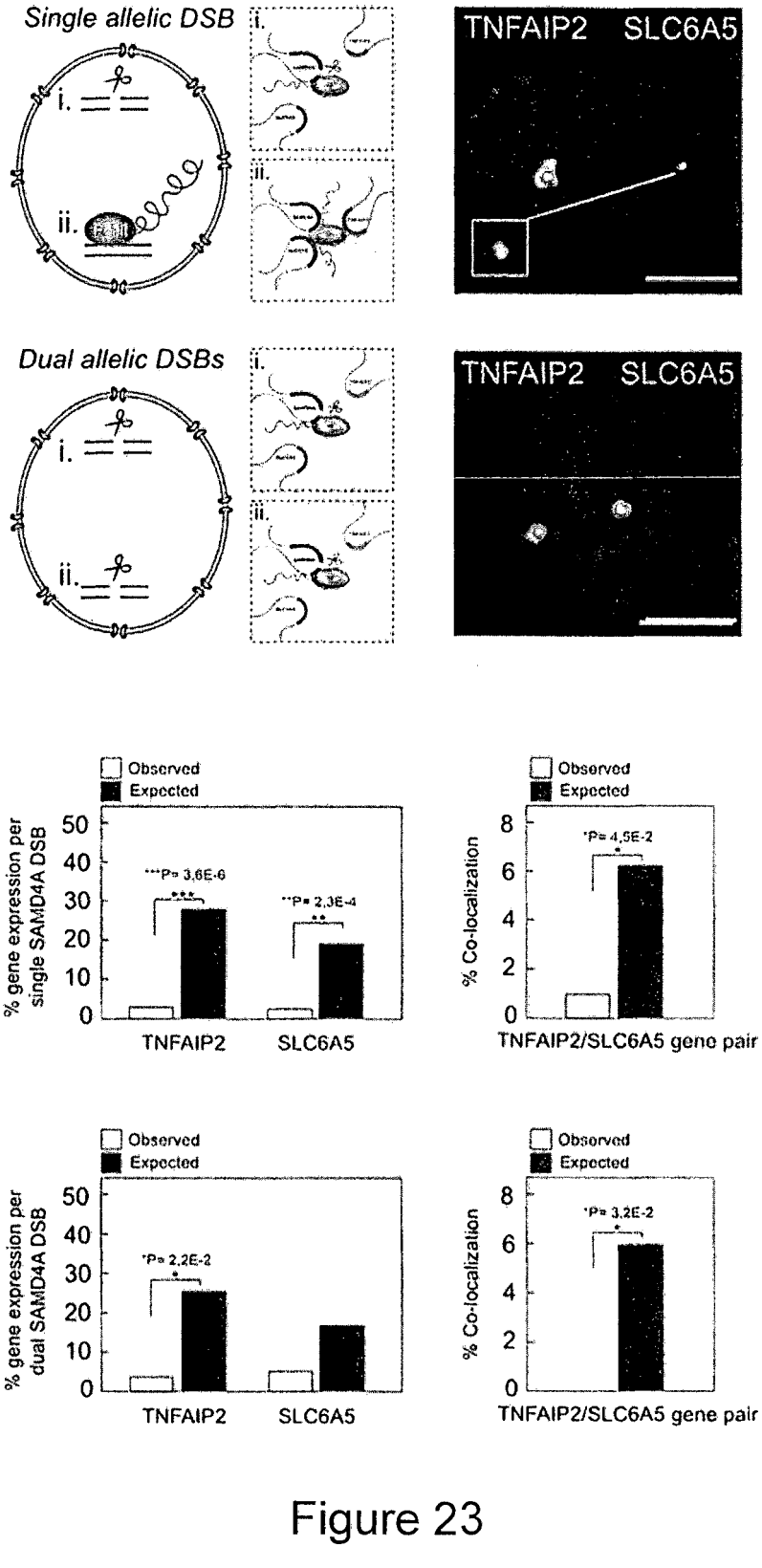

Single allelic DSB
i.
ii.
TNFAIP2 SLC6A5
Dual allelic DSBs
Observed
Expected
% gene expression per single SAMD4A DSB
***P= 3,6E-6
**P= 2,3E-4
TNFAIP2
SLC6A5
% Co-localization
*P= 4,5E-2
TNFAIP2/SLC6A5 gene pair
% gene expression per dual SAMD4A DSB
*P= 2,2E-2
*P= 3,2E-2

Figure 23

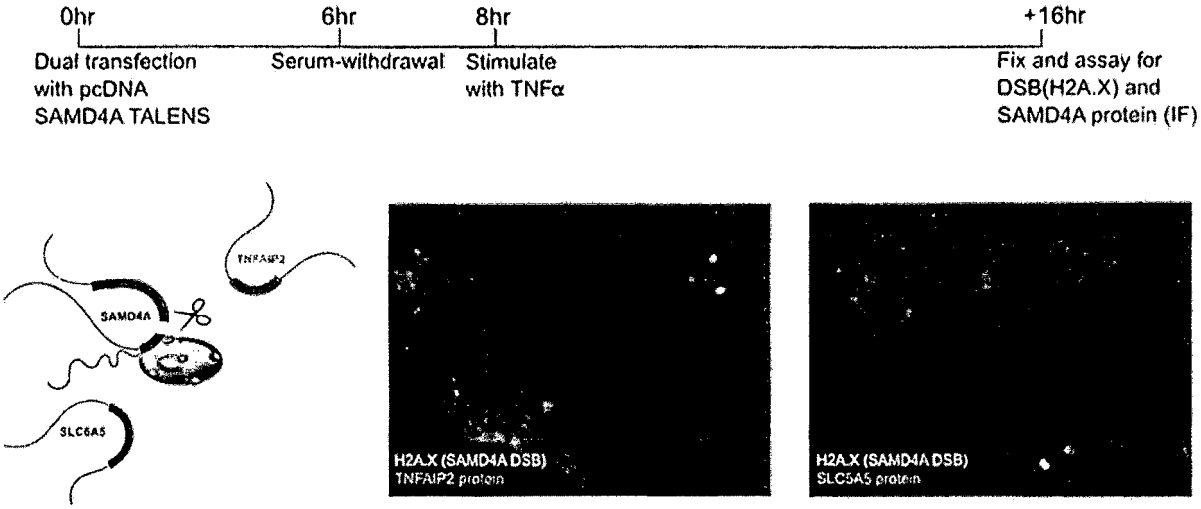


Figure 24

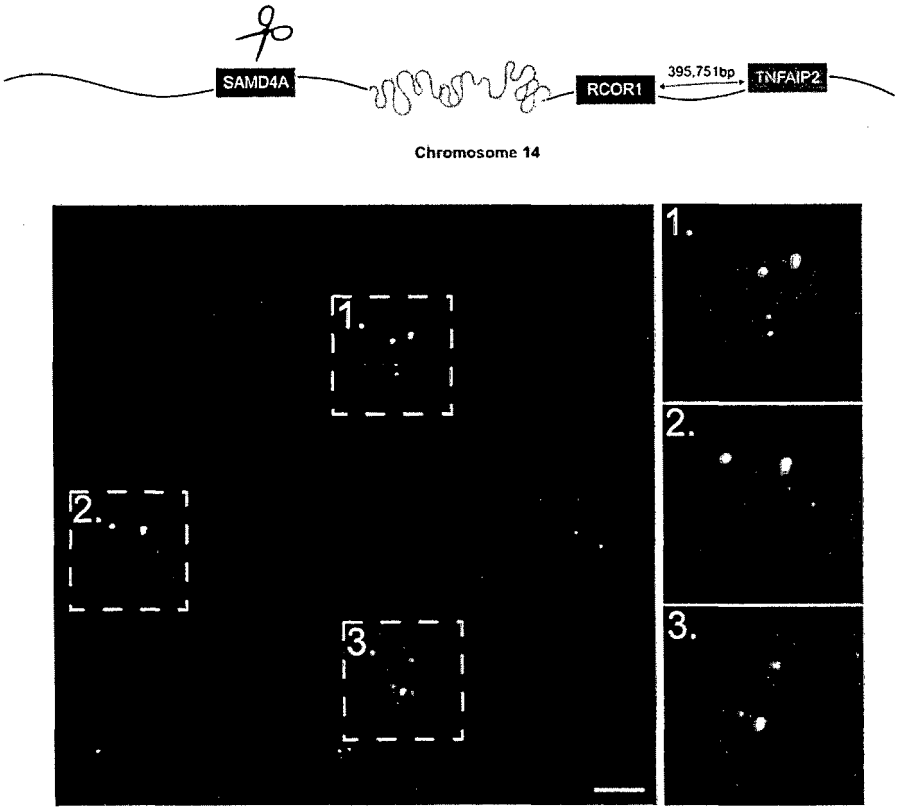


Figure 25

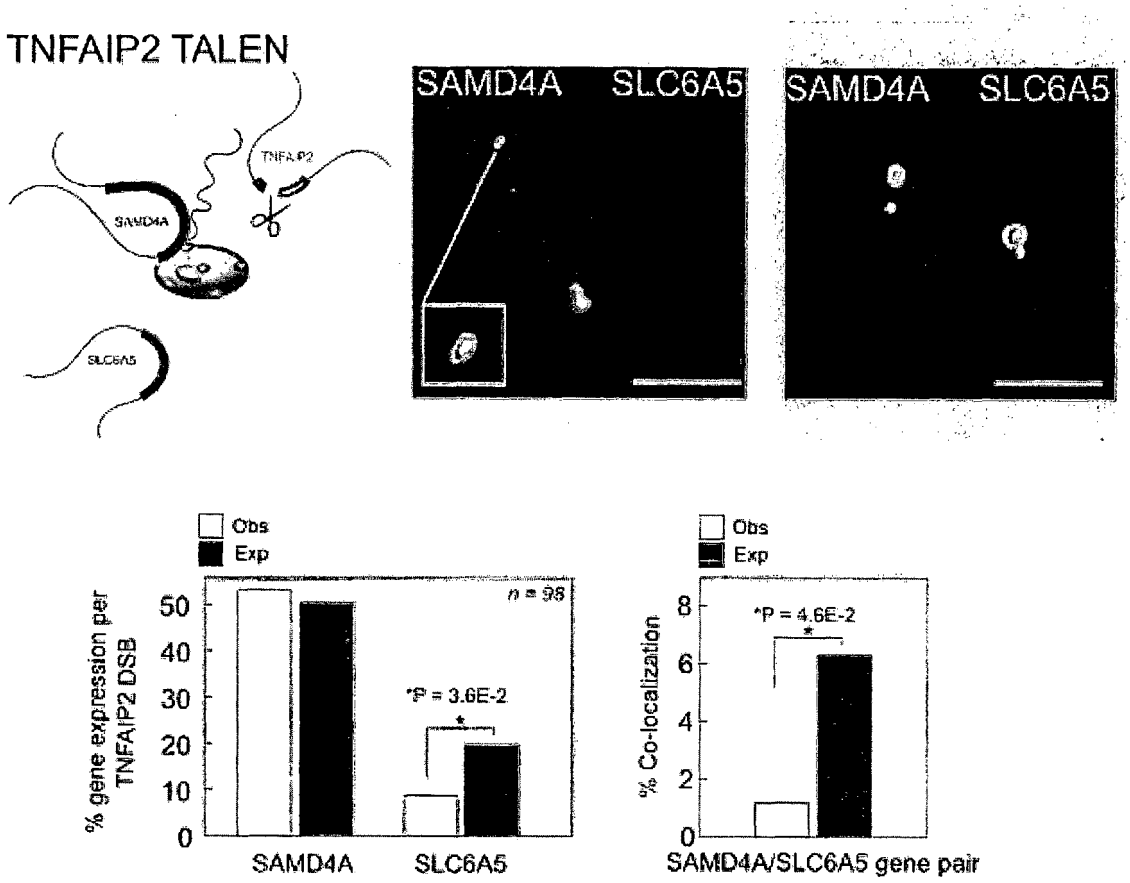


Figure 26

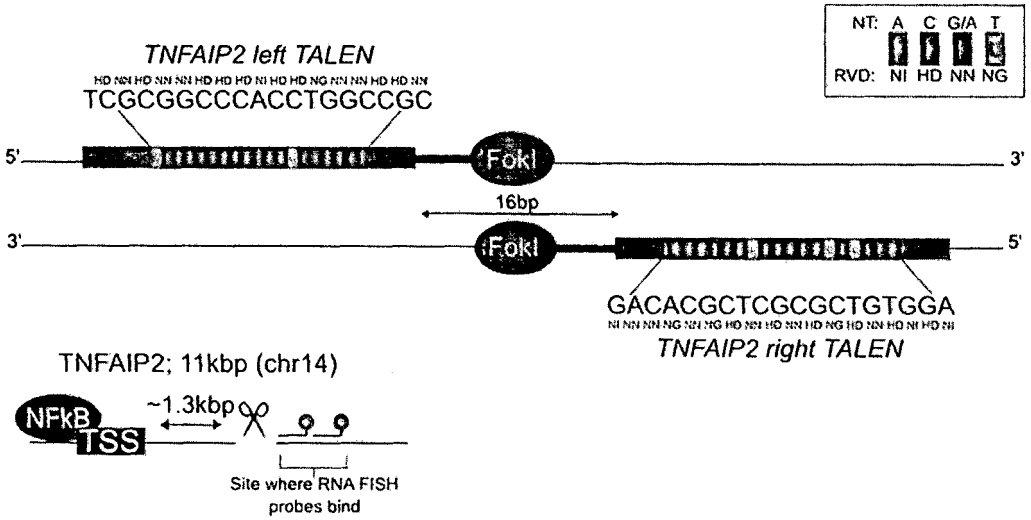


Figure 27

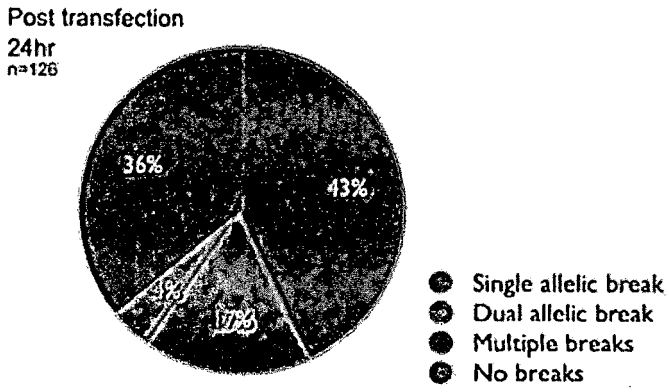


Figure 28

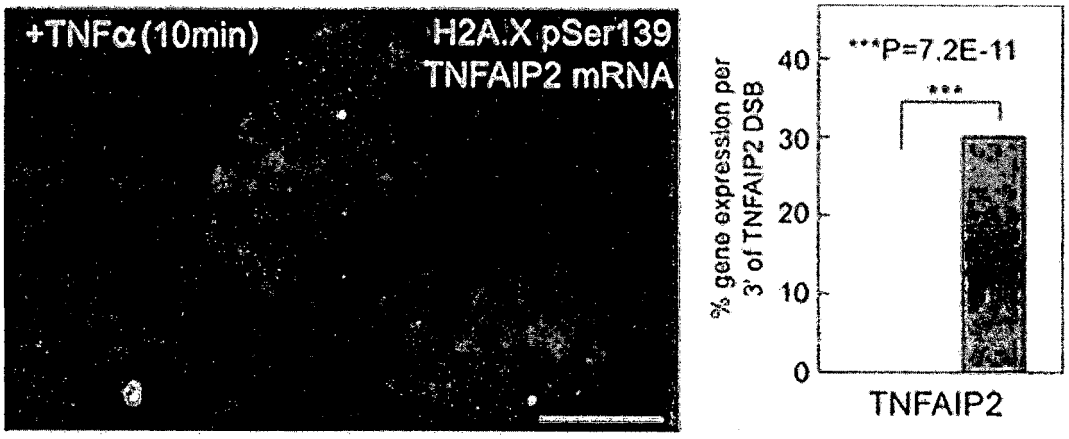


Figure 29

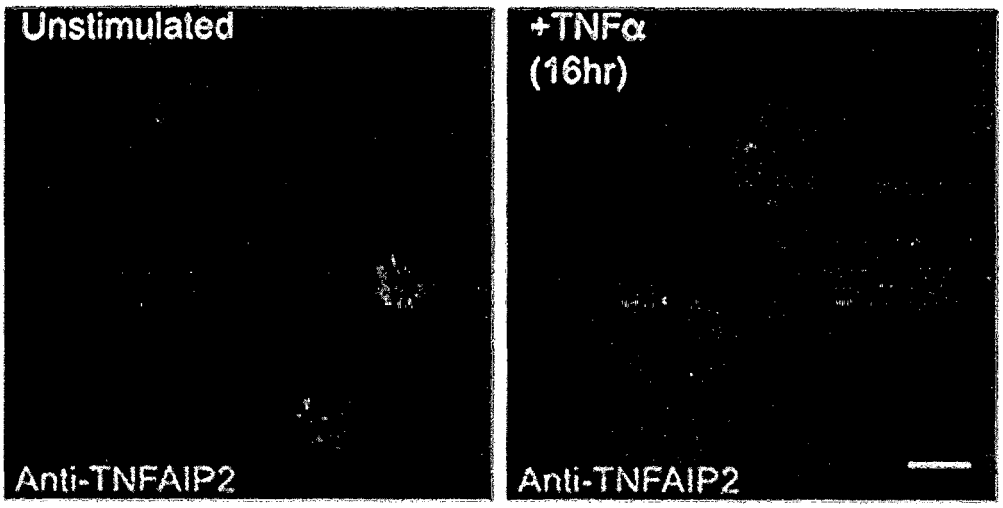


Figure 30

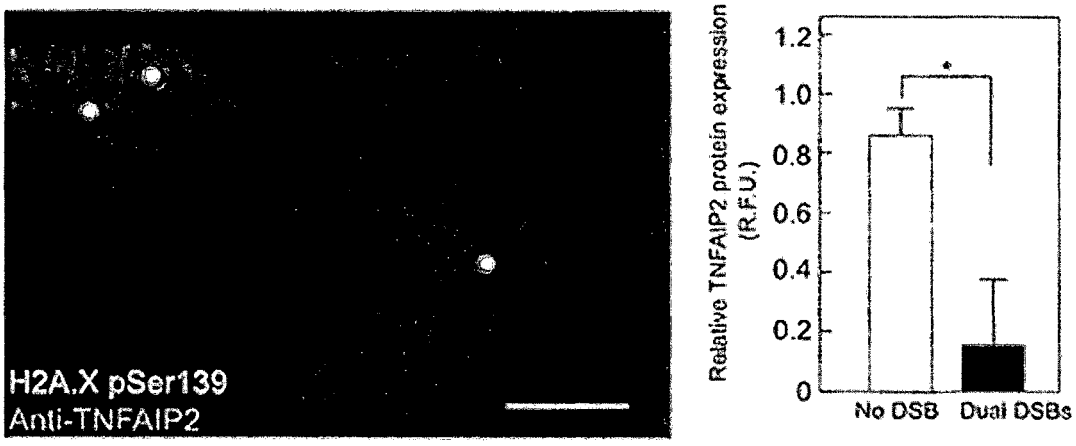


Figure 31

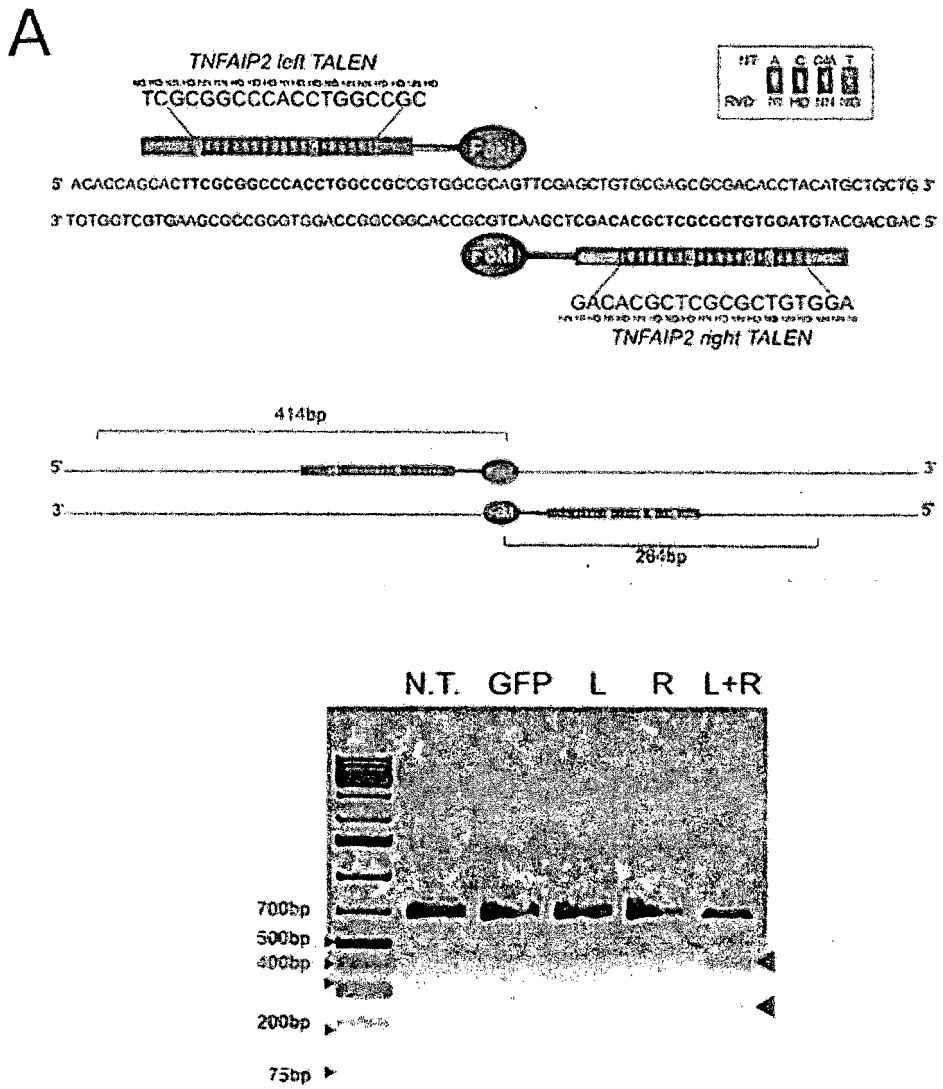

A
TNFAIP2 left TALEN
TCGCGGCCCACCTGGCCGC
FokI
5' ACACCAGCACTTCGCGGCCCACCTGGCCGCCGTGGCGCAGTTCGAGCTGTGCGAGCGCGACACCTACATGCTGCTG 3'
3' TGTGGTCGTGAAGCGCCGGGTGGACCGGCGGCACCGCGTCAAGCTCGACACGCTCGCGCTGTGGATGTACGACGAC 5'
FokI
GACACGCTCGCGCTGTGGA
TNFAIP2 right TALEN
414bp
5'
3'
3'
5'
264bp
N.T. GFP L R L+R
700bp
500bp
400bp
200bp
75bp

Figure 32

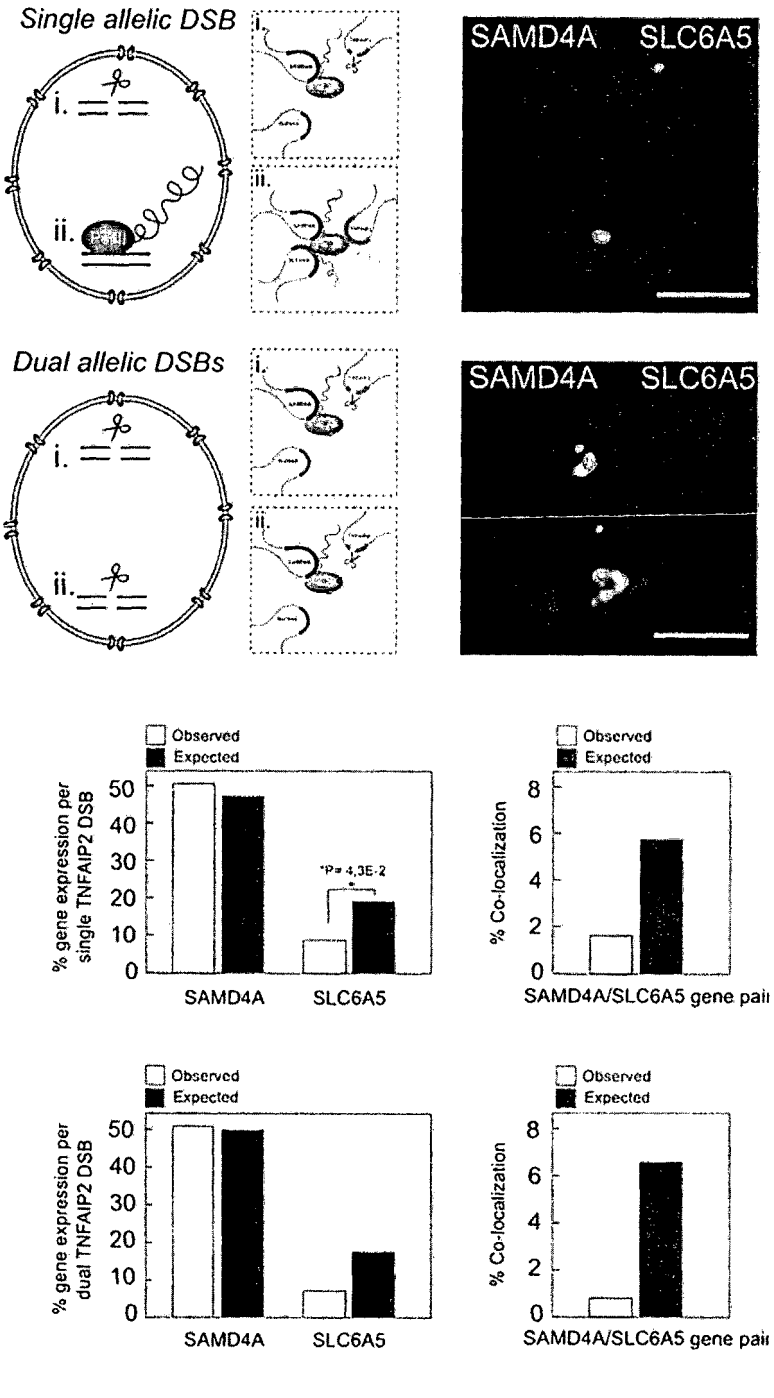

Single allelic DSB
i.
ii.
SAMD4A SLC6A5
Dual allelic DSBs
i.
ii.
SAMD4A SLC6A5
Observed
Expected
% gene expression per single TNFAIP2 DSB
50
40
30
20
10
0
*P= 4,3E-2
SAMD4A
SLC6A5
% Co-localization
8
6
4
2
0
SAMD4A/SLC6A5 gene pair
Observed
Expected
% gene expression per dual TNFAIP2 DSB
50
40
30
20
10
0
SAMD4A
SLC6A5
% Co-localization
8
6
4
2
0
SAMD4A/SLC6A5 gene pair

Figure 33

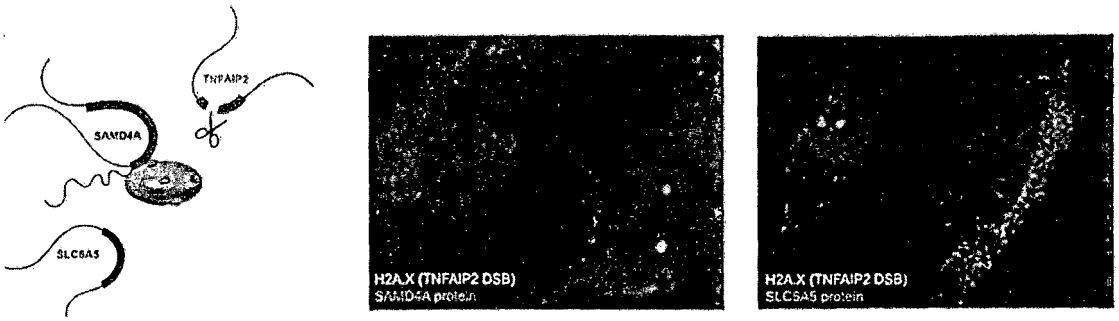


Figure 34

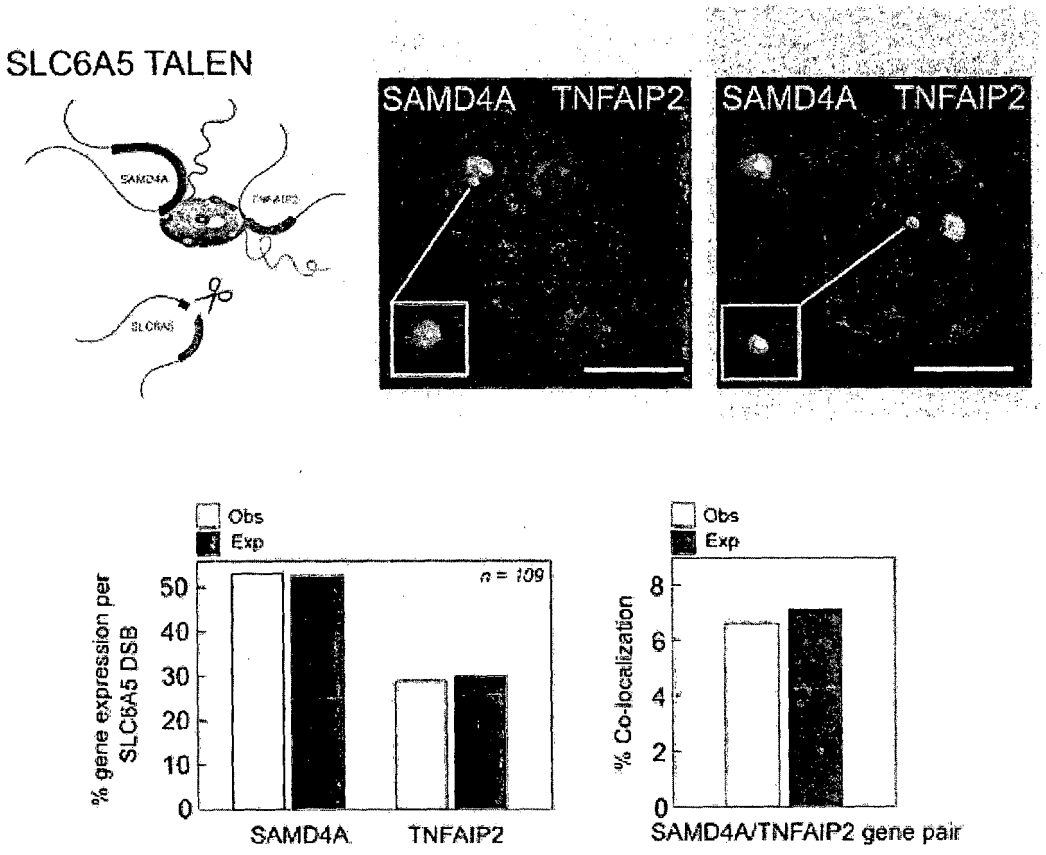


Figure 35

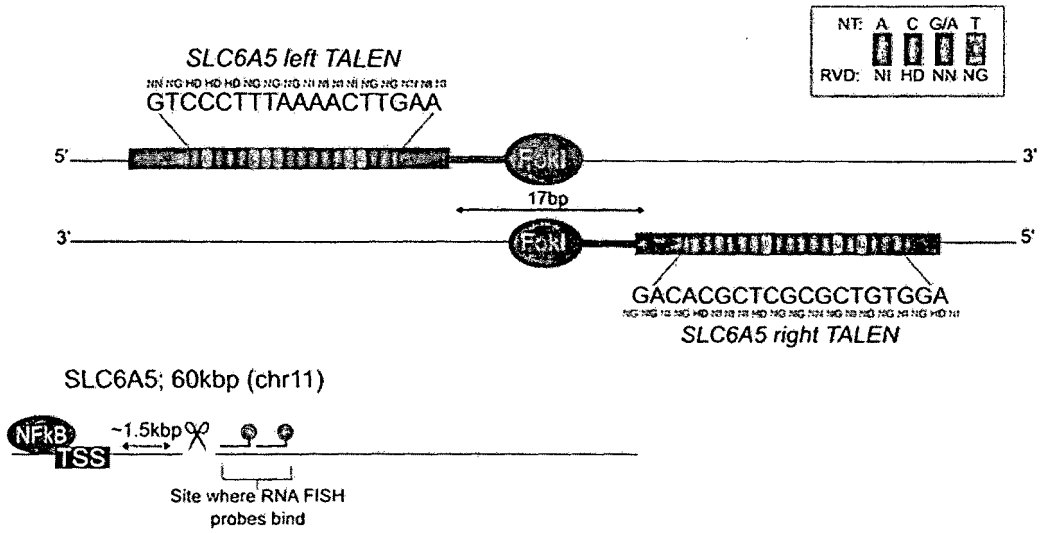


Figure 36

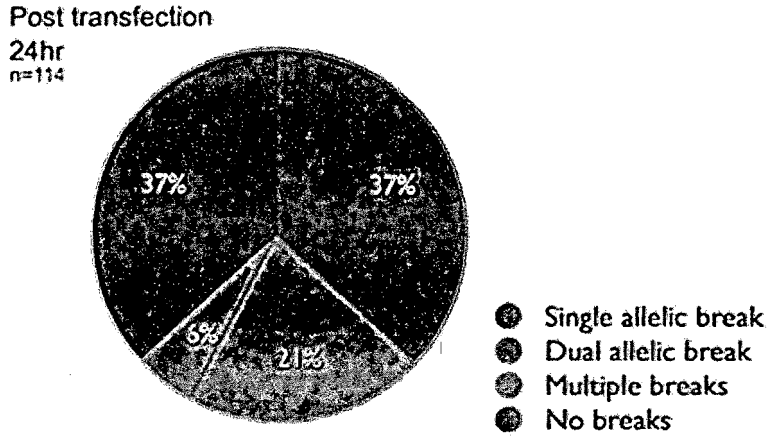


Figure 37

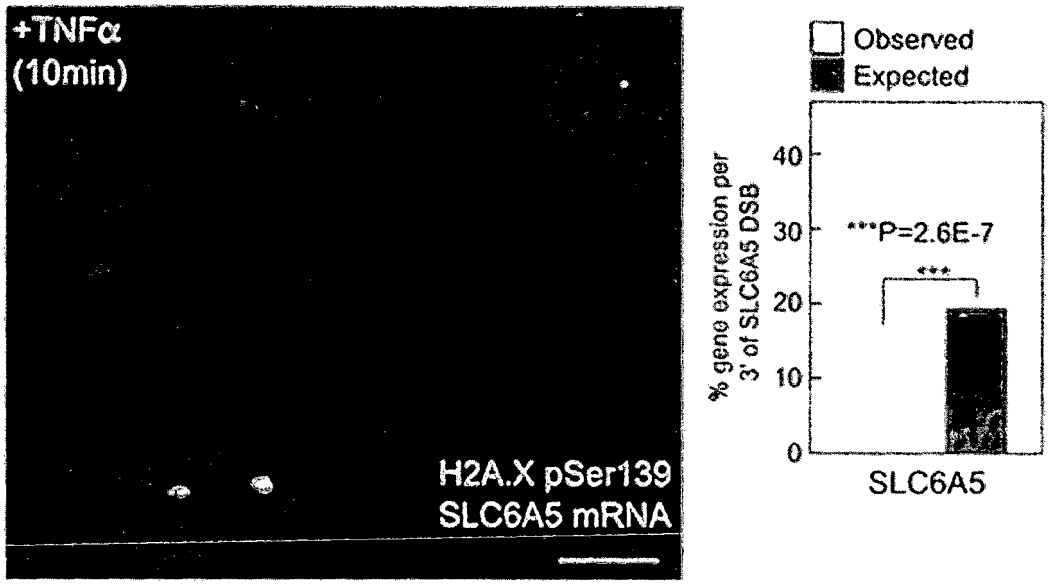


Figure 38

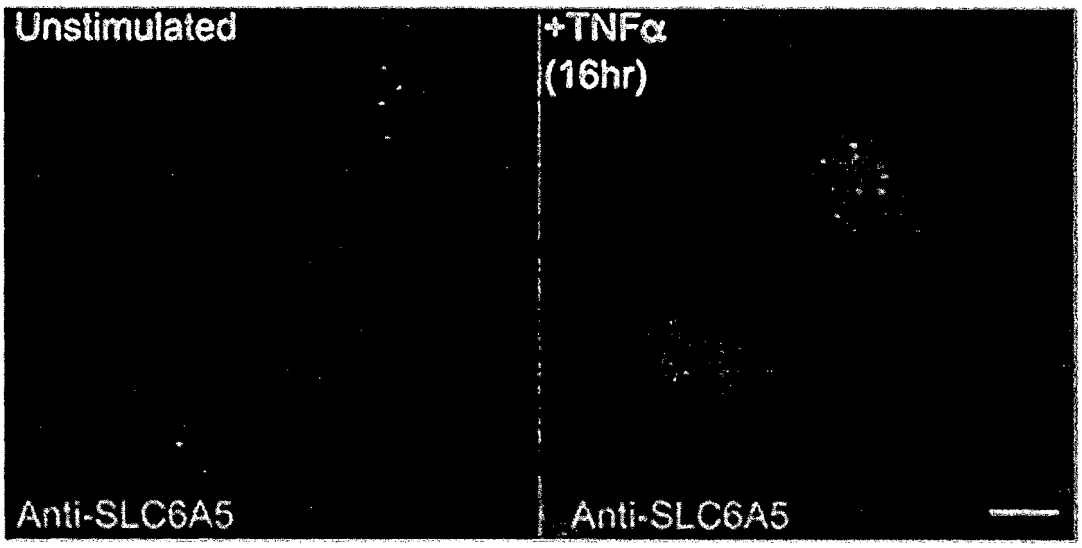


Figure 39

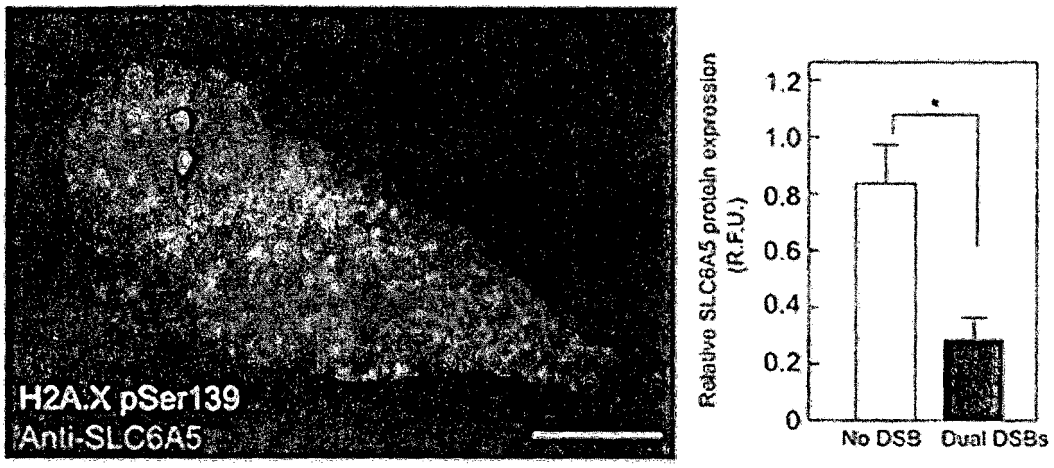


Figure 40

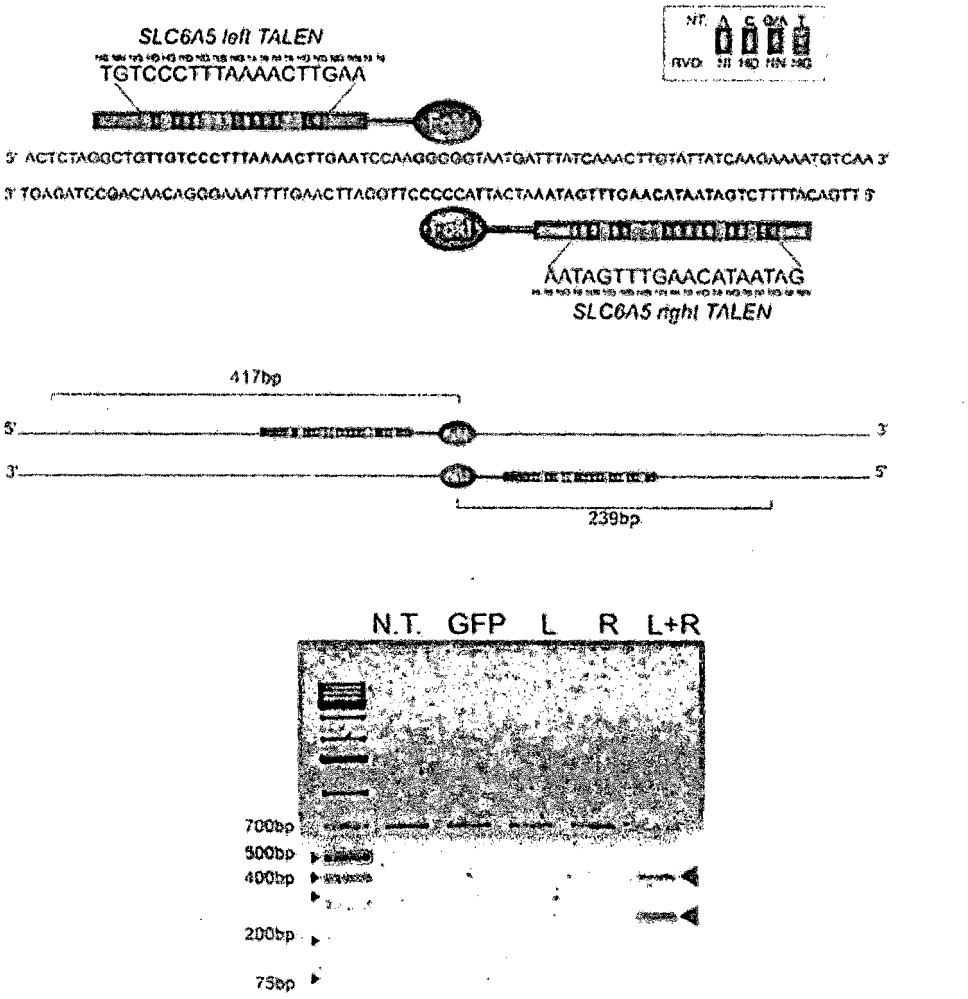


Figure 41

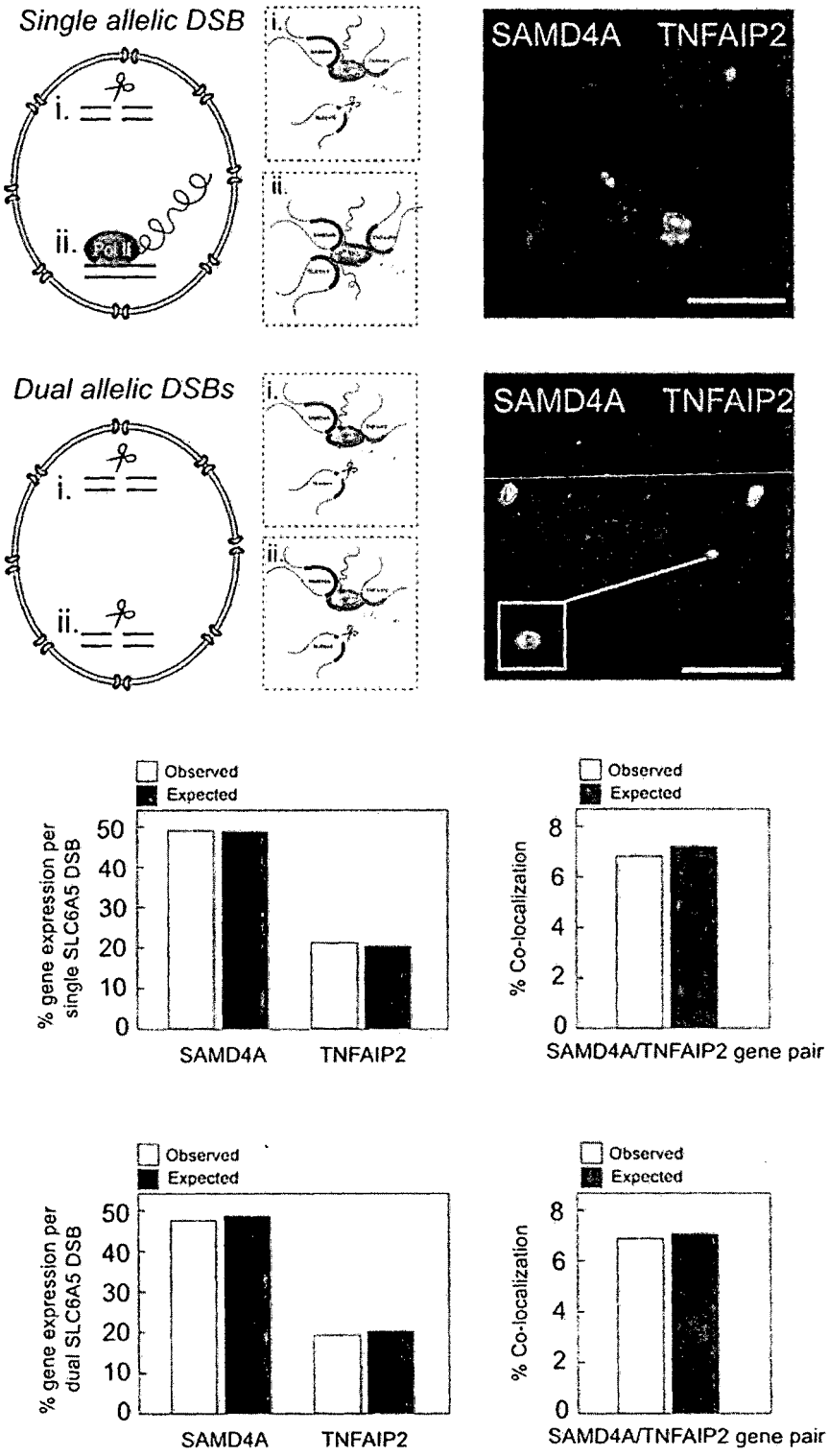

Single allelic DSB
i.
ii.
Pol II
SAMD4A TNFAIP2
Dual allelic DSBs
% gene expression per single SLC6A5 DSB
Observed
Expected
SAMD4A
TNFAIP2
% Co-localization
SAMD4A/TNFAIP2 gene pair
% gene expression per dual SLC6A5 DSB

Figure 42

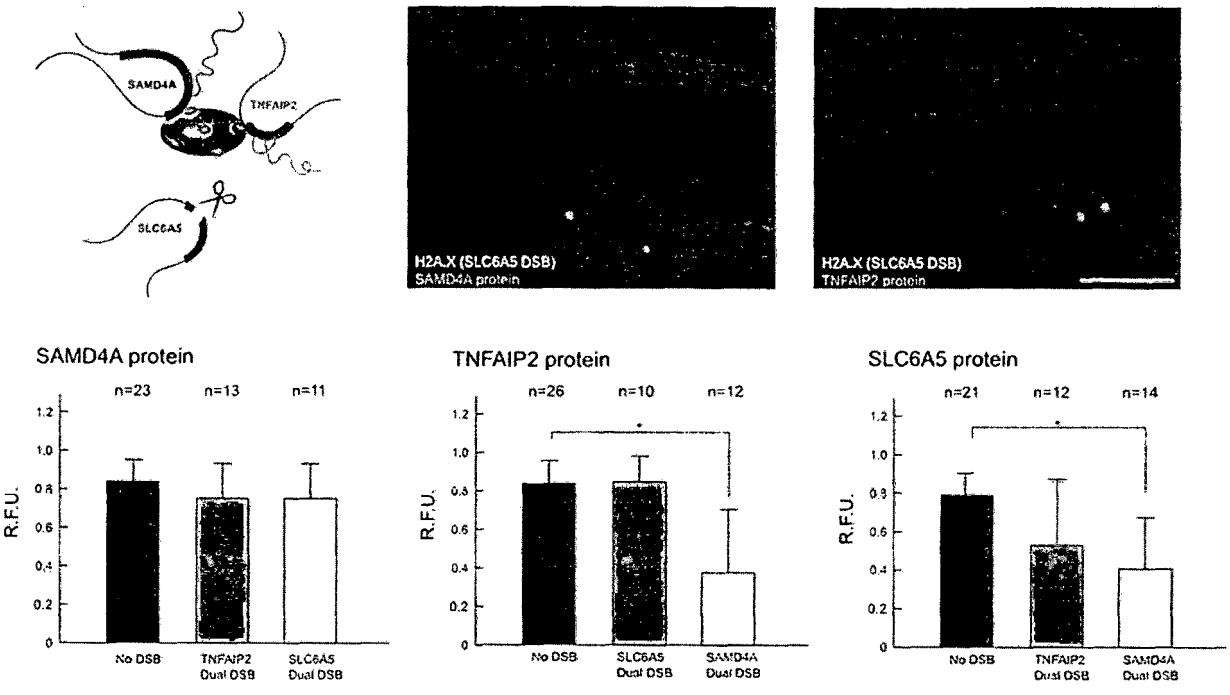

SAMD4A
TNFAIP2
SLC6A5
H2A.X (SLC6A5 DSB)
SAMD4A protein
H2A.X (SLC6A5 DSB)
TNFAIP2 protein
SAMD4A protein
n=23
n=13
n=11
R.F.U.
No DSB
TNFAIP2 Dual DSB
SLC6A5 Dual DSB
TNFAIP2 protein
n=26
n=10
n=12
No DSB
SLC6A5 Dual DSB
SAMD4A Dual DSB
SLC6A5 protein
n=21
n=12
n=14
No DSB
TNFAIP2 Dual DSB
SAMD4A Dual DSB

Figure 43

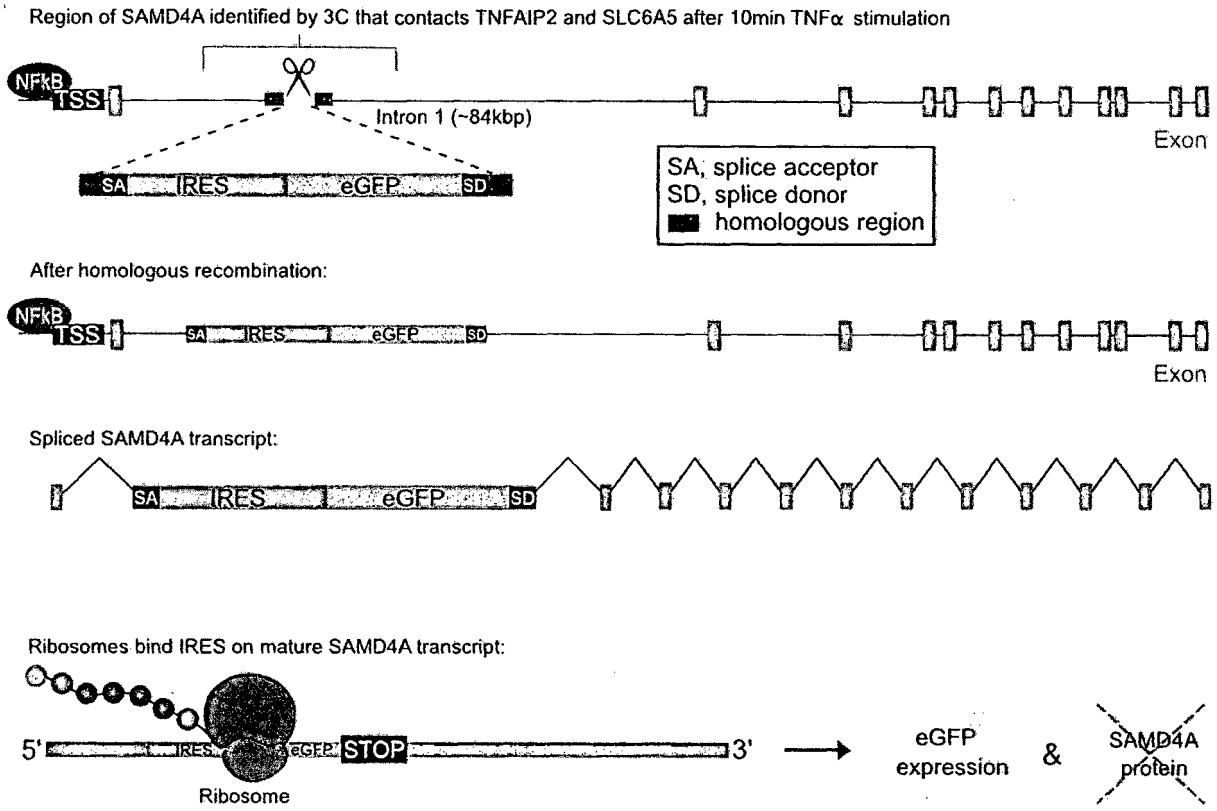

Region of SAMD4A identified by 3C that contacts TNFAIP2 and SLC6A5 after 10min TNFα stimulation
NFkB
TSS
Intron 1 (~84kbp)
Exon
SA
IRES
eGFP
SD
SA, splice acceptor
SD, splice donor
homologous region
After homologous recombination:
NFkB
TSS
SA
IRES
eGFP
SD
Exon
Spliced SAMD4A transcript:
SA
IRES
eGFP
SD
Ribosomes bind IRES on mature SAMD4A transcript:
5'
IRES
eGFP
STOP
3'
Ribosome
eGFP expression
&
SAMD4A protein

Figure 44

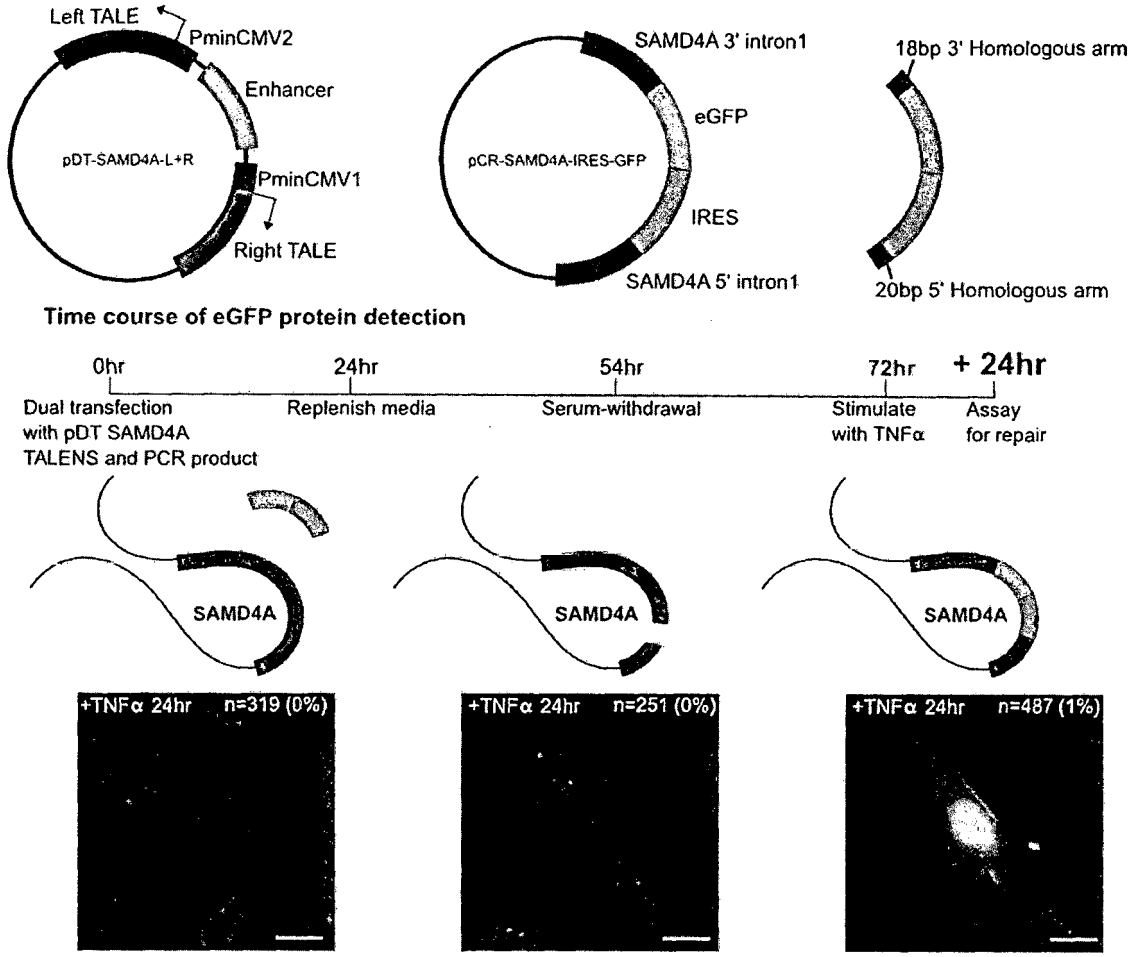

Left TALE
PminCMV2
Enhancer
pDT-SAMD4A-L+R
PminCMV1
Right TALE
SAMD4A 3' intron1
eGFP
pCR-SAMD4A-IRES-GFP
IRES
SAMD4A 5' intron1
18bp 3' Homologous arm
20bp 5' Homologous arm
Time course of eGFP protein detection
0hr
24hr
54hr
72hr
+ 24hr
Dual transfection with pDT SAMD4A TALENS and PCR product
Replenish media
Serum-withdrawal
Stimulate with TNFα
Assay for repair
SAMD4A
SAMD4A
SAMD4A
+TNFα 24hr n=319 (0%)
+TNFα 24hr n=251 (0%)
+TNFα 24hr n=487 (1%)

Figure 45

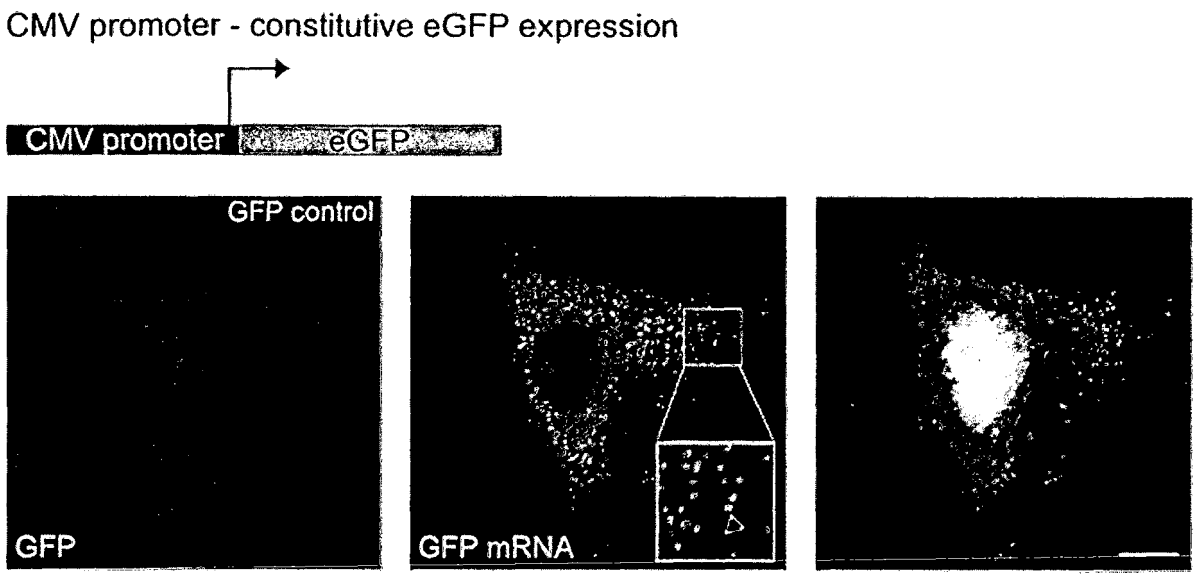


Figure 46

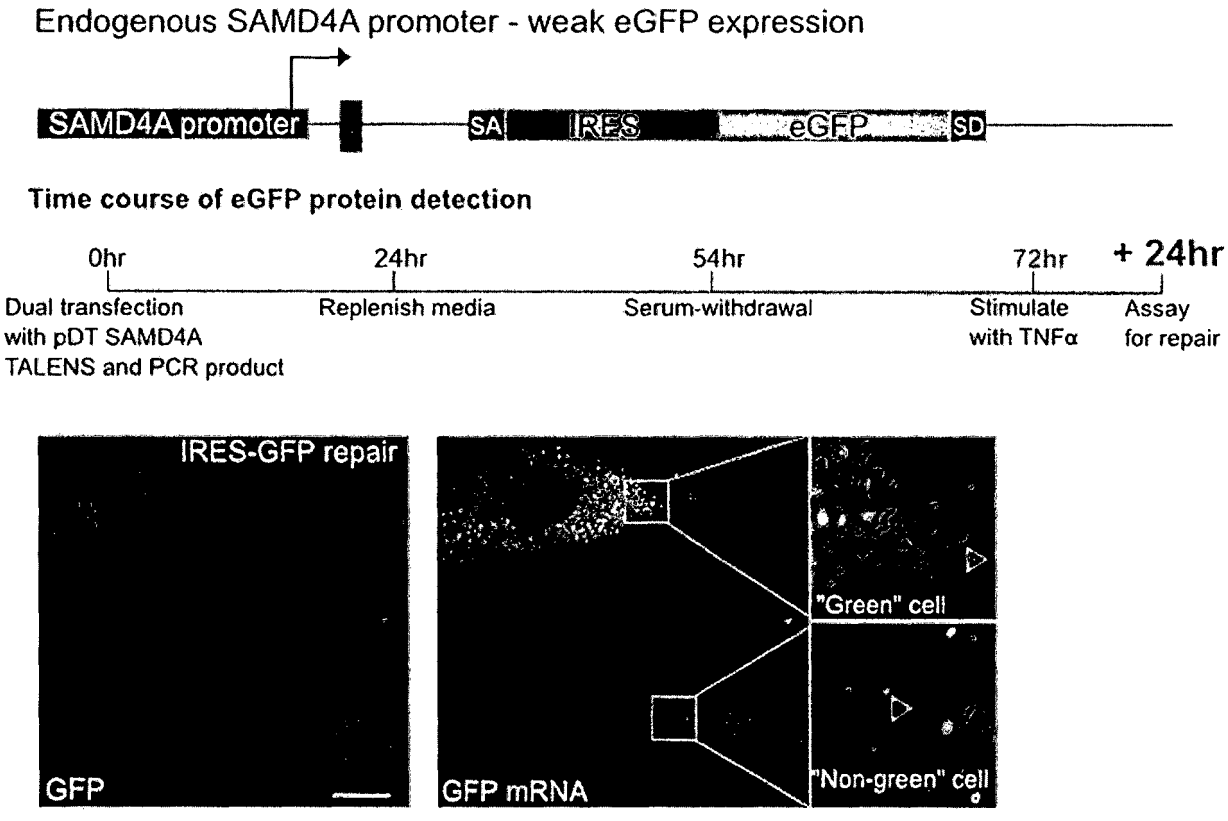


Figure 47

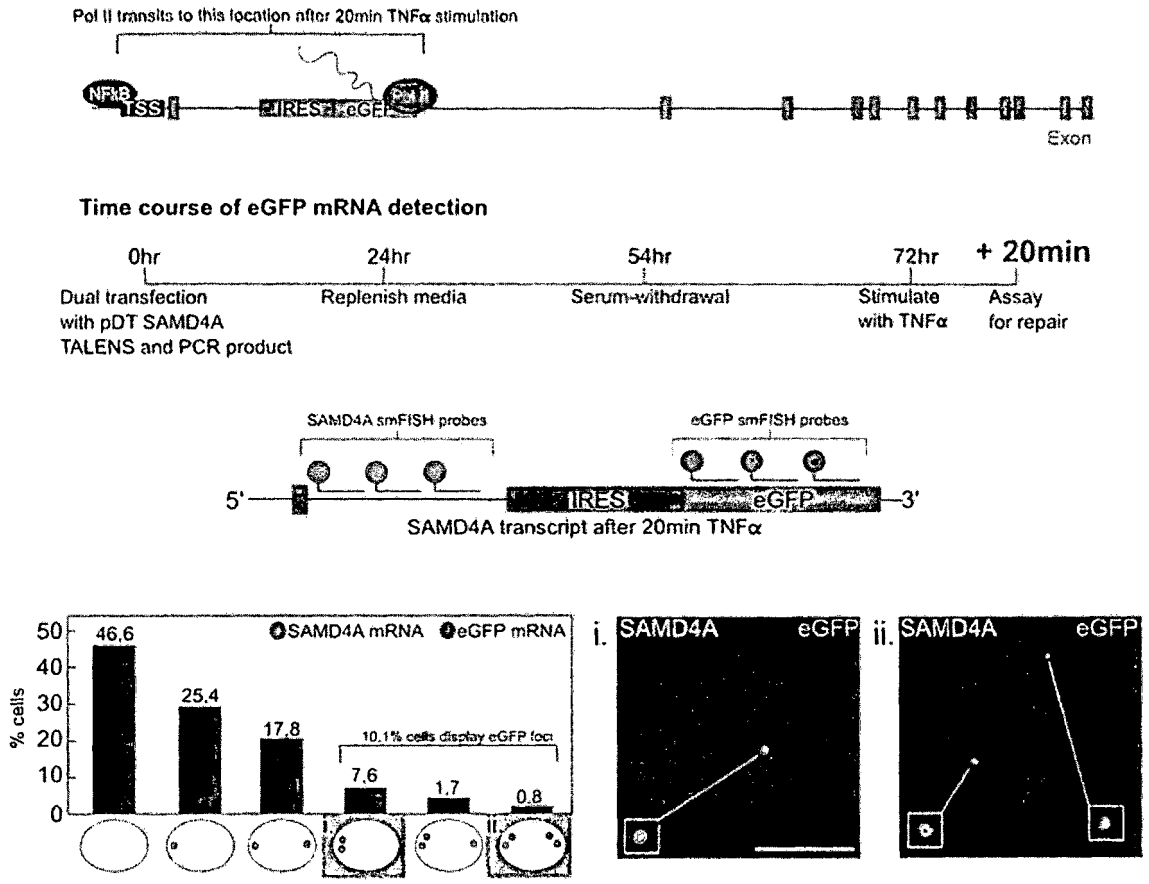


Figure 48

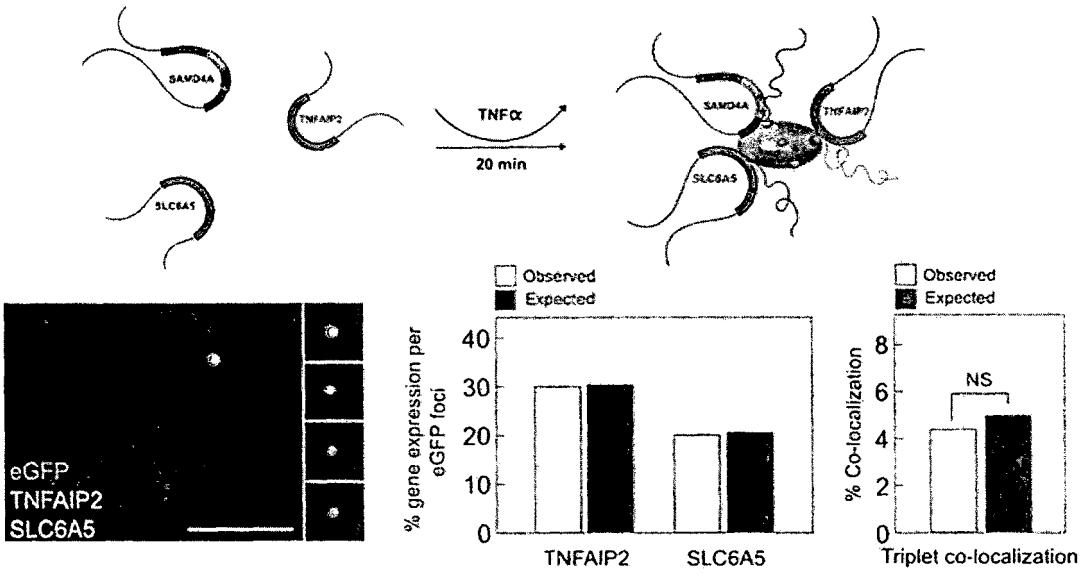


Figure 49

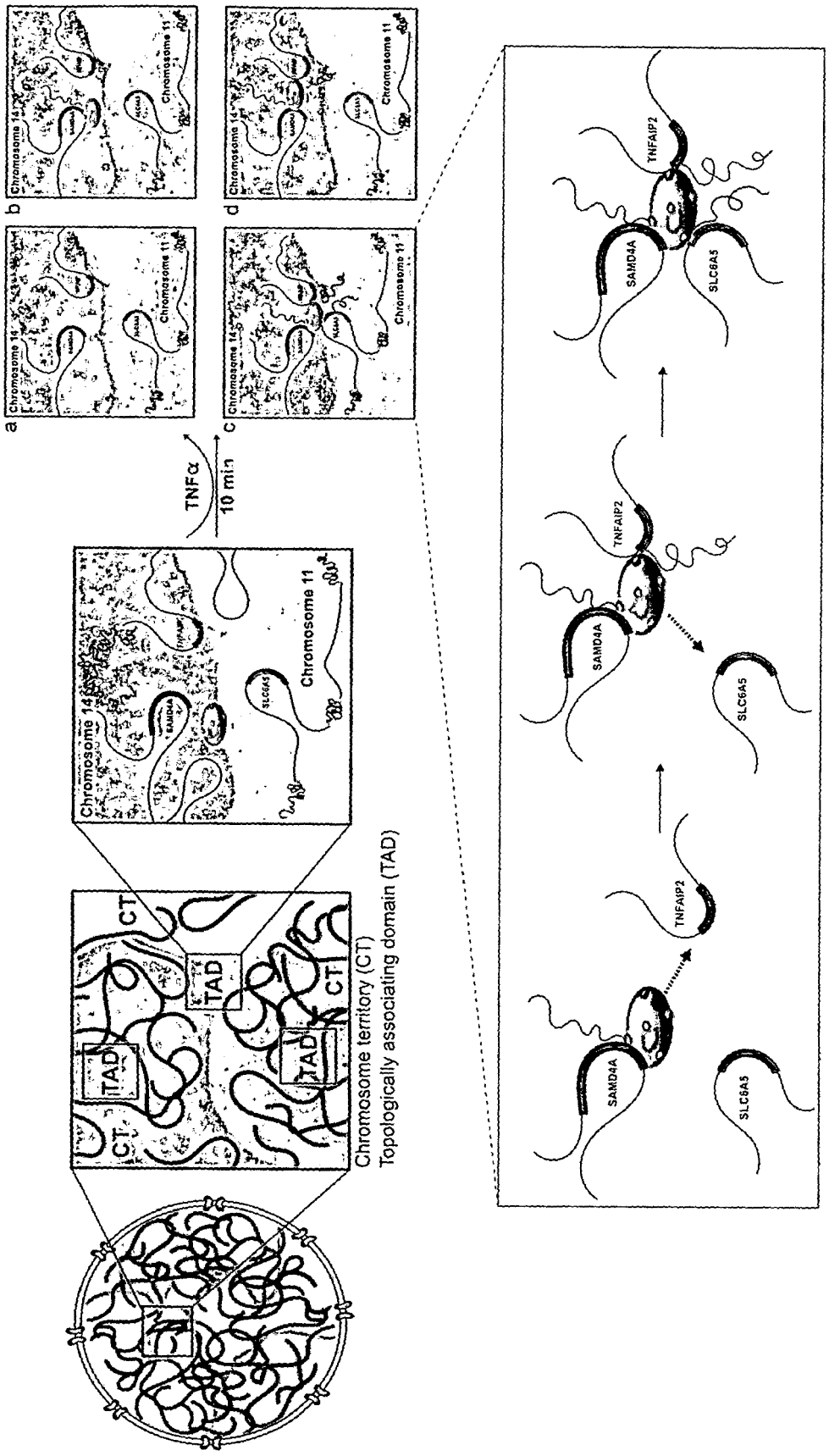

a
b
c
d
Chromosome 14
Chromosome 11
TNFα
10 min
CT
TAD
Chromosome territory (CT)
Topologically associating domain (TAD)
SAMD4A
TNFAIP2
SLC6A5

Figure 50

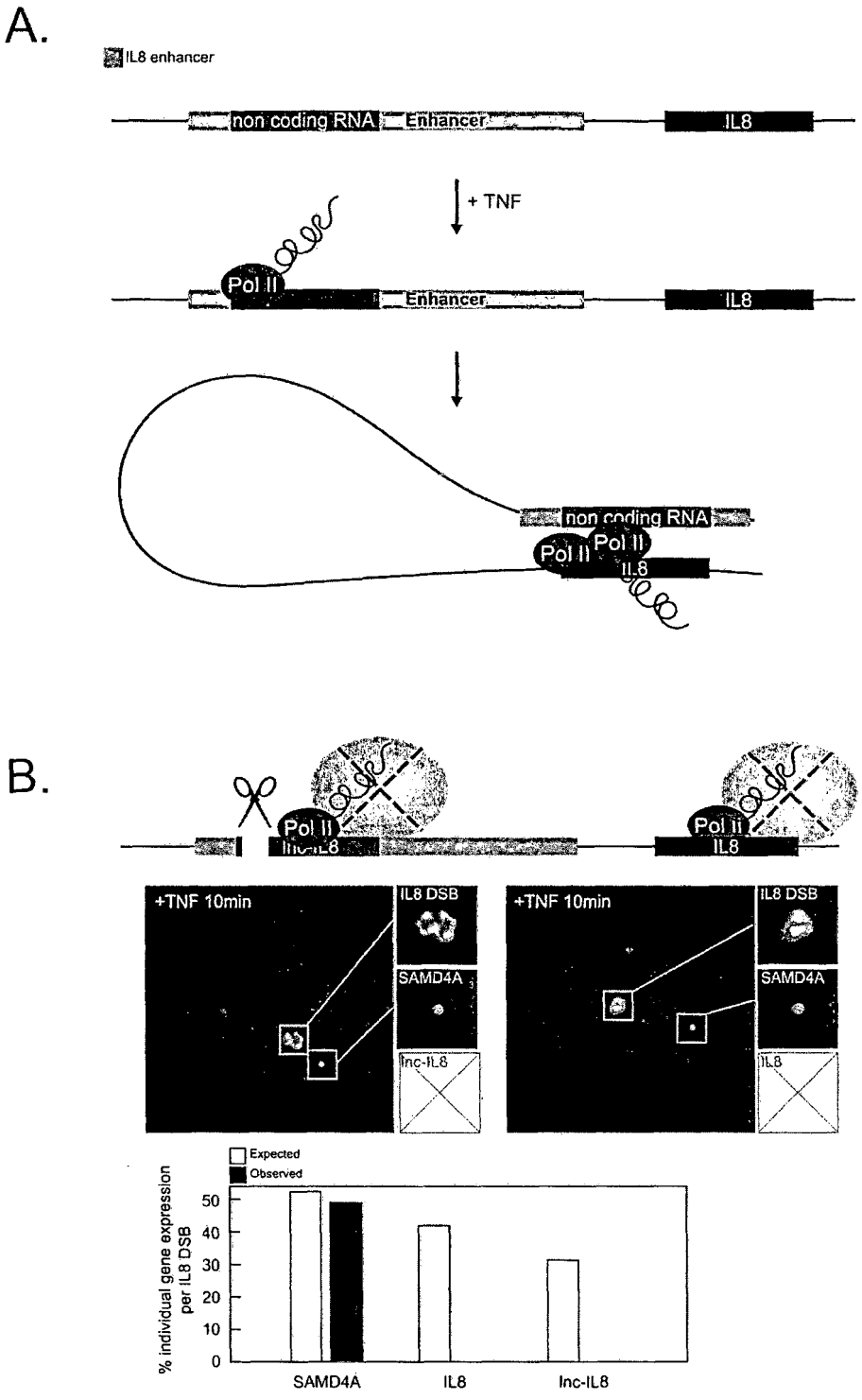

A.
IL8 enhancer
non coding RNA
Enhancer
IL8
+ TNF
Pol II
Enhancer
IL8
non coding RNA
Pol II
Pol II
IL8
B.
Pol II
lnc-IL8
Pol II
IL8
+TNF 10min
IL8 DSB
SAMD4A
lnc-IL8
+TNF 10min
IL8 DSB
SAMD4A
IL8
Expected
Observed
% individual gene expression per IL8 DSB
50
40
30
20
10
0
SAMD4A
IL8
lnc-IL8

Figure 51

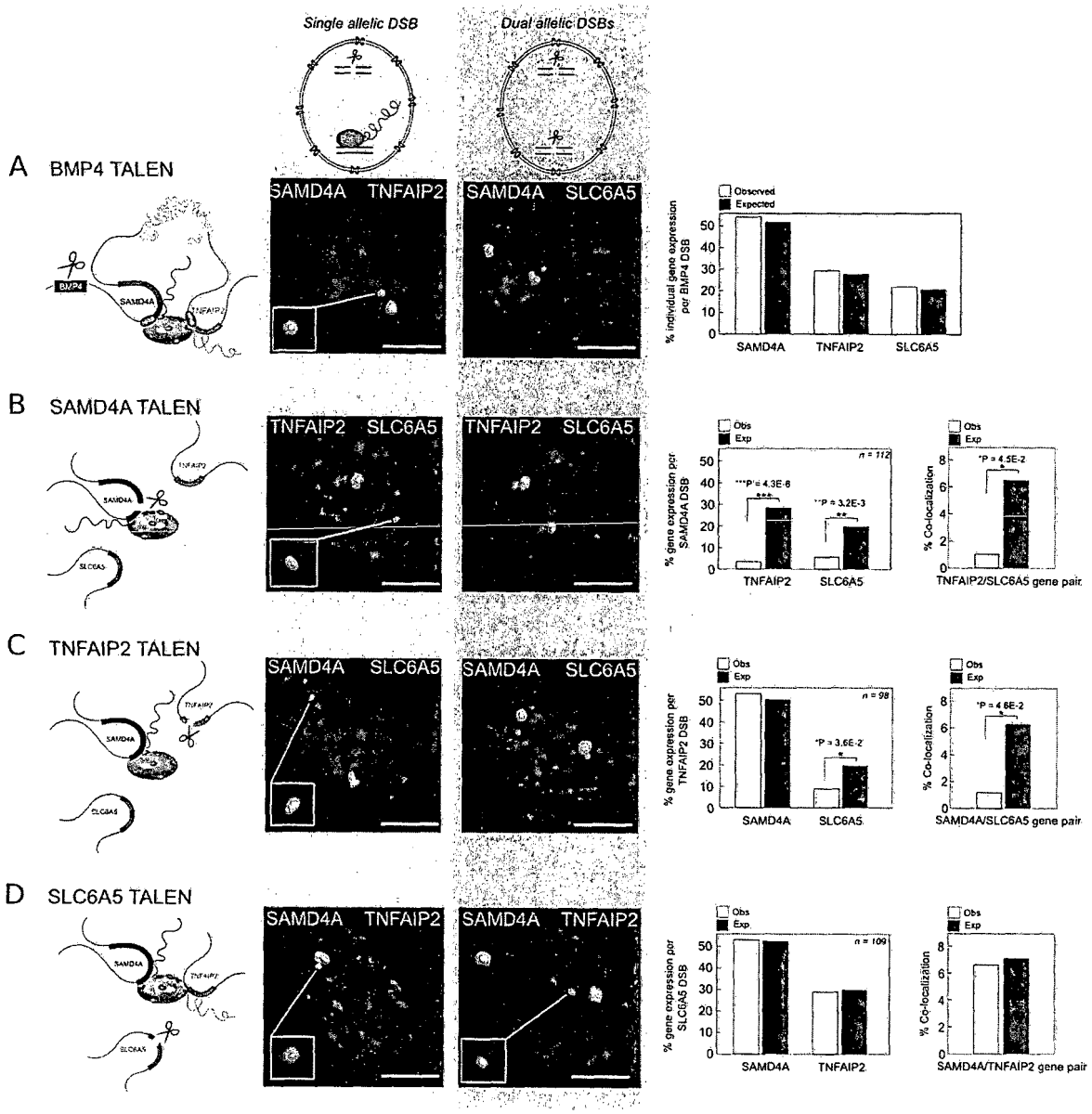

Single allelic DSB
Dual allelic DSBs
A BMP4 TALEN
SAMD4A TNFAIP2
SAMD4A SLC6A5
Observed
Expected
% individual gene expression per BMP4 DSB
SAMD4A
TNFAIP2
SLC6A5
B SAMD4A TALEN
TNFAIP2 SLC6A5
TNFAIP2 SLC6A5
Obs
Exp
% gene expression per SAMD4A DSB
n = 112
***P = 4.3E-6
**P = 3.2E-3
TNFAIP2
SLC6A5
% Co-localization
*P = 4.5E-2
TNFAIP2/SLC6A5 gene pair
C TNFAIP2 TALEN
SAMD4A SLC6A5
SAMD4A SLC6A5
% gene expression per TNFAIP2 DSB
n = 98
*P = 3.6E-2
*P = 4.6E-2
SAMD4A/SLC6A5 gene pair
D SLC6A5 TALEN
SAMD4A TNFAIP2
SAMD4A TNFAIP2
% gene expression per SLC6A5 DSB
n = 109
SAMD4A/TNFAIP2 gene pair

Figure 52

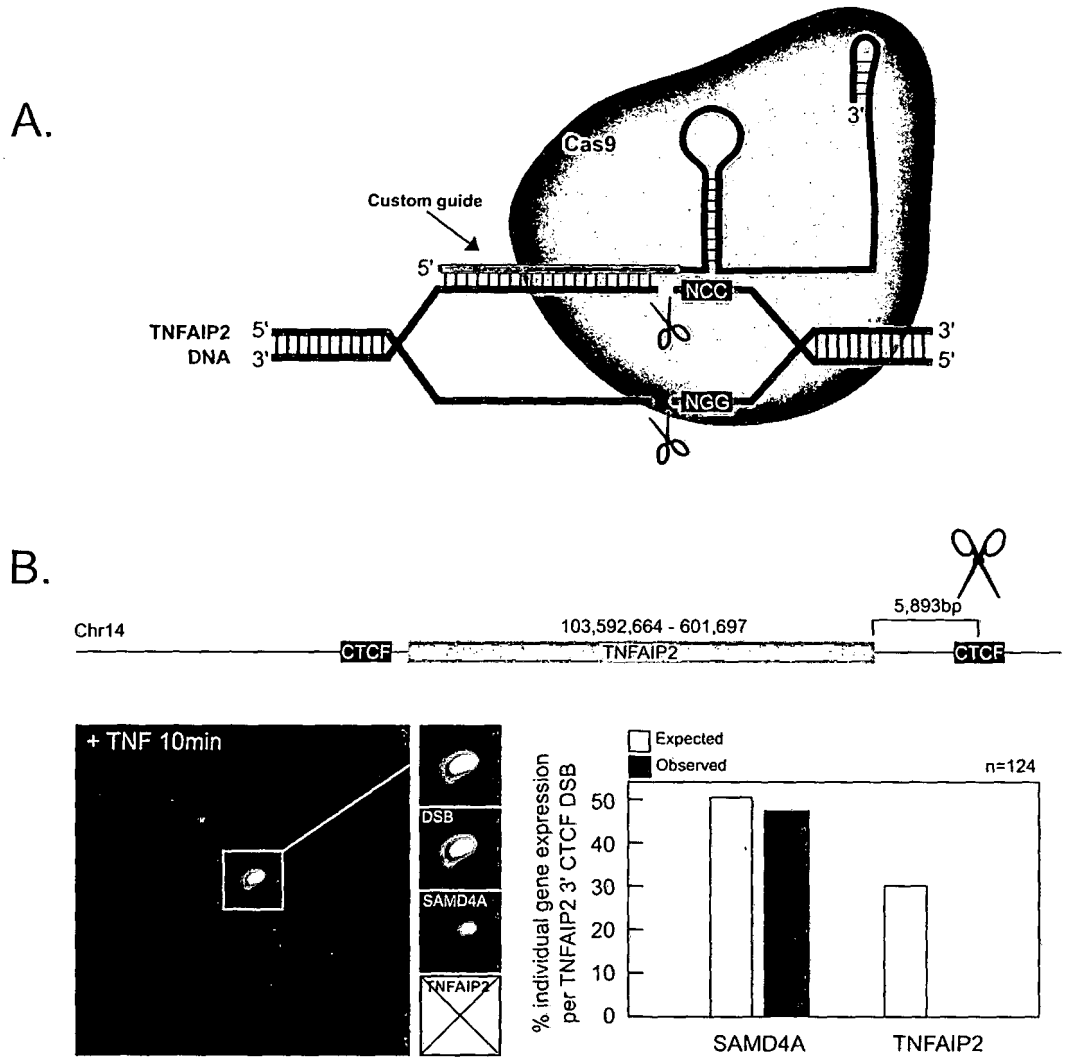

A.
Cas9
Custom guide
5'
3'
NCC
NGG
TNFAIP2 5'
DNA 3'
3'
5'
B.
5,893bp
Chr14
103,592,664 - 601,697
CTCF
TNFAIP2
CTCF
+ TNF 10min
DSB
SAMD4A
TNFAIP2
Expected
Observed
n=124
% individual gene expression per TNFAIP2 3' CTCF DSB
50
40
30
20
10
0
SAMD4A
TNFAIP2

Figure 53