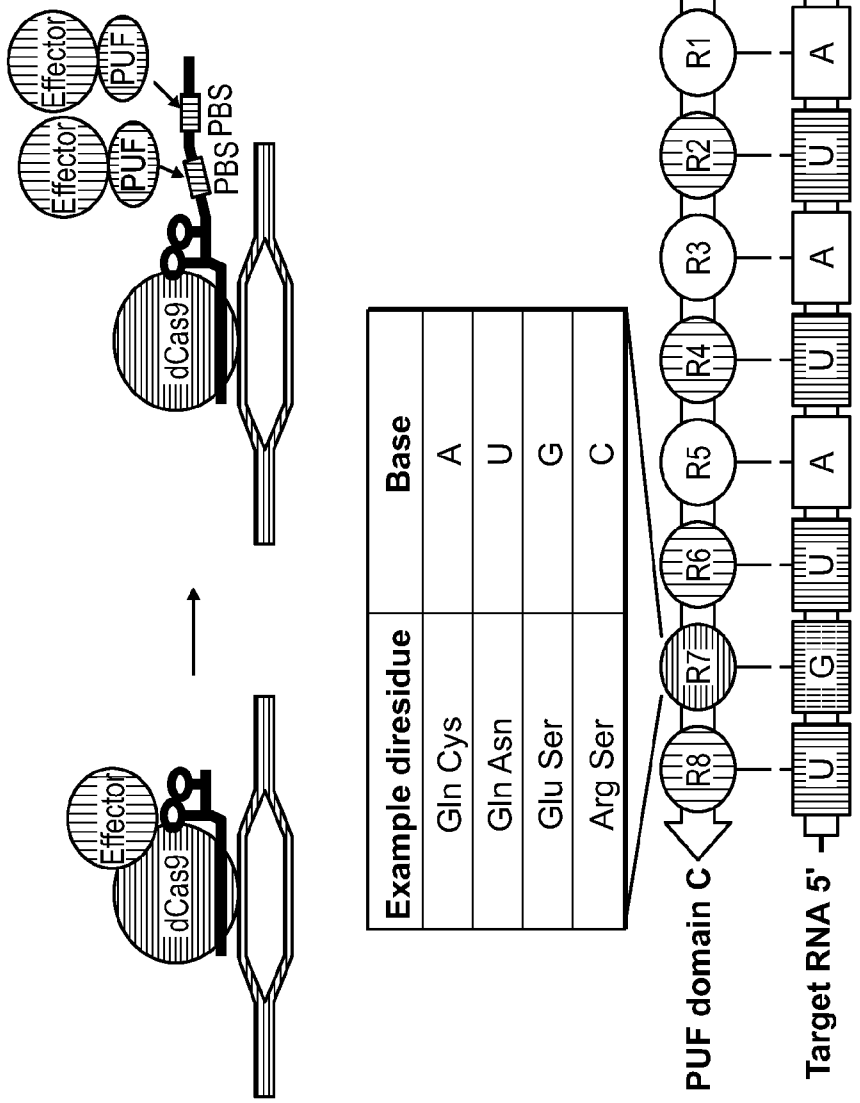
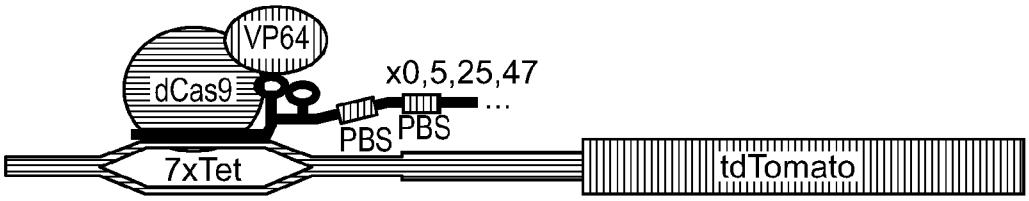


Example diresidue	Base
Gln Cys	A
Gln Asn	U
Glu Ser	G
Arg Ser	C

Literature			Simplified		Binding Sequence
PUF type	PBS type	Ref	PUF type	PBS type	
PUF(3-2)	A6G	[?]	PUFa	PBSa	UUGAU**g**UA
PUF(6-2/7-2)	GU/UG	[?]	PUFb	PBSb	U**ug**AUAUA
PUF(WT)	NRE	[?]	PUFw	PBSw	UGUAUAUA
-	-	-	PUFc	PBSc	U**ug**AU**g**UA

FIG. 1A

FIG. 1B


VP64
dCas9
x0,5,25,47
...
PBS
PBS
7xTet
tdTomato

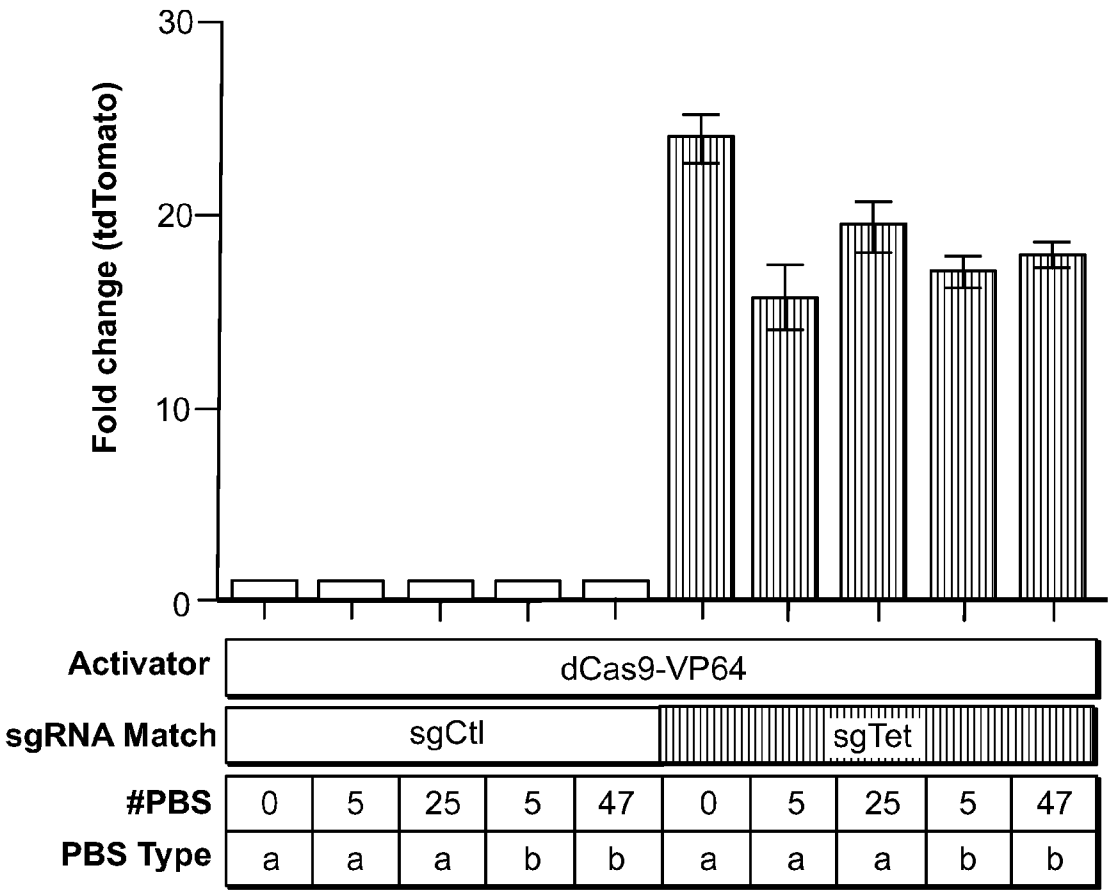

30
20
10
0
Fold change (tdTomato)
Activator
dCas9-VP64
sgRNA Match
sgCtl
sgTet
#PBS
0 5 25 5 47 0 5 25 5 47
PBS Type
a a a b b a a a b b

FIG. 1C

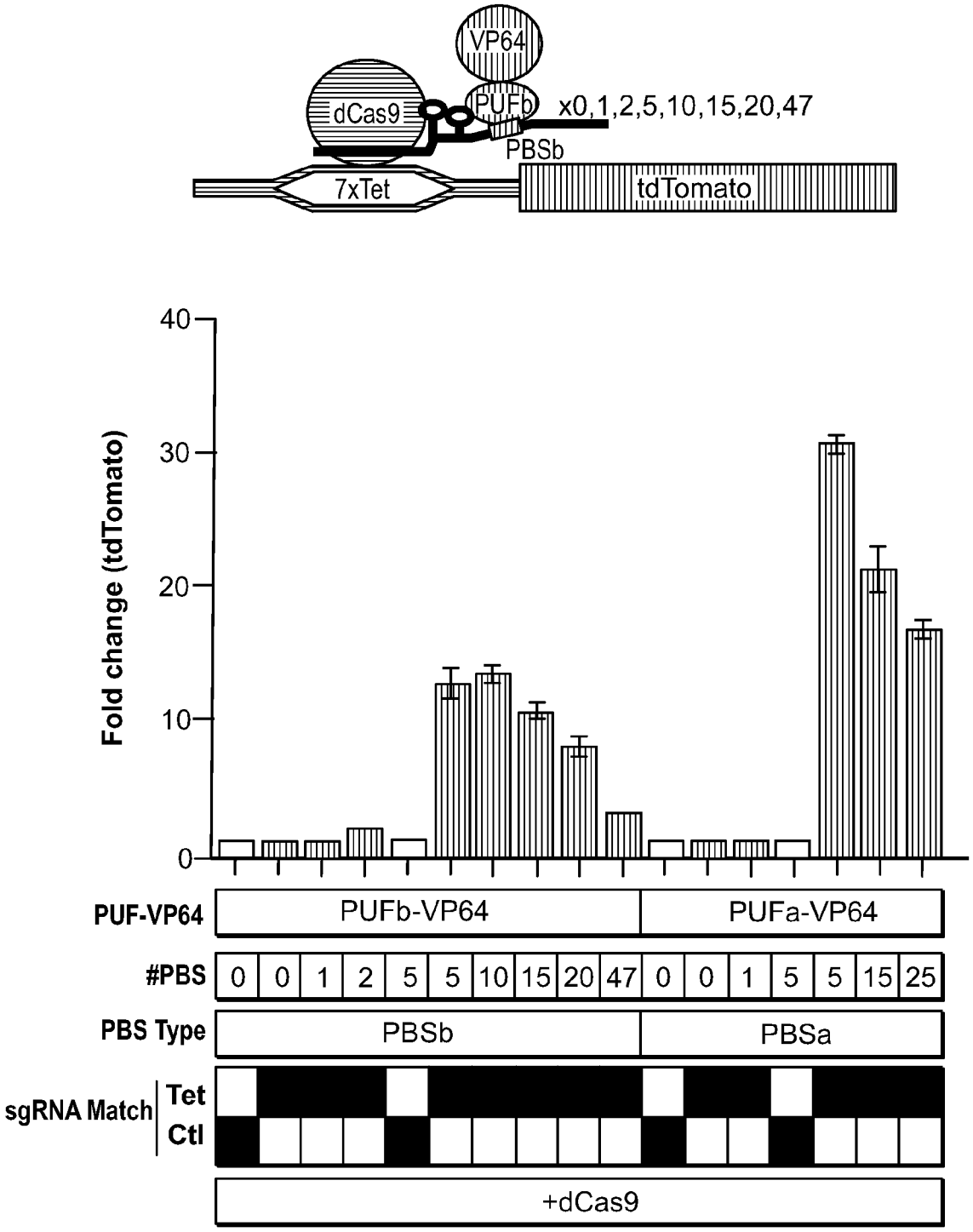

VP64
dCas9
PUFb
x0,1,2,5,10,15,20,47
PBSb
7xTet
tdTomato
Fold change (tdTomato)
40
30
20
10
0
PUF-VP64
PUFb-VP64
PUFa-VP64
#PBS
0 0 1 2 5 5 10 15 20 47 0 0 1 5 5 15 25
PBS Type
PBSb
PBSa
sgRNA Match
Tet
Ctl
+dCas9

FIG. 1D

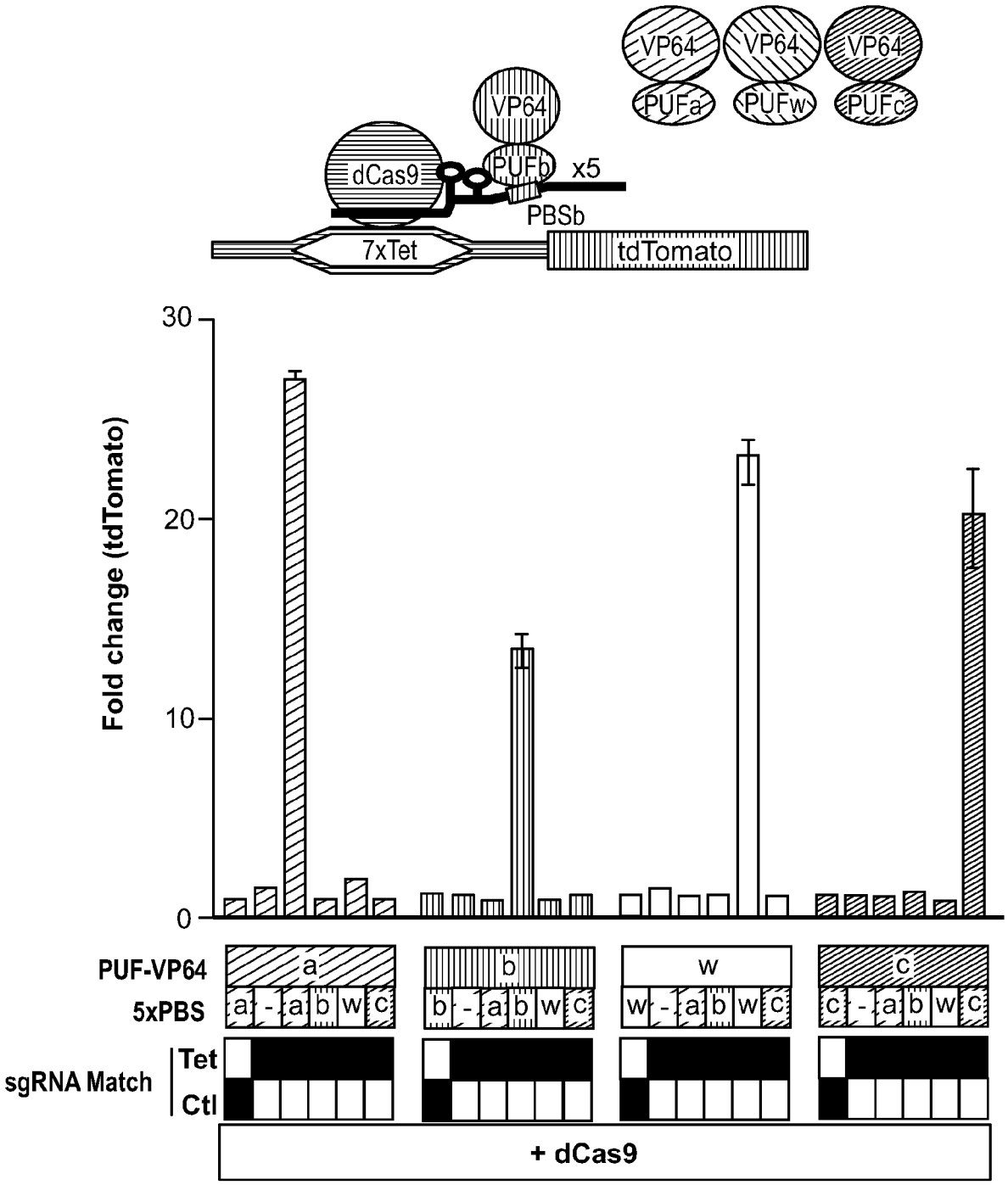

VP64
VP64
VP64
PUFa
PUFw
PUFc
VP64
dCas9
PUFb
x5
PBSb
7xTet
tdTomato
30
20
10
0
Fold change (tdTomato)
PUF-VP64
5xPBS
a
b
w
c
a - a b w c
b - a b w c
w - a b w c
c - a b w c
sgRNA Match
Tet
Ctl
+ dCas9

FIG. 2A

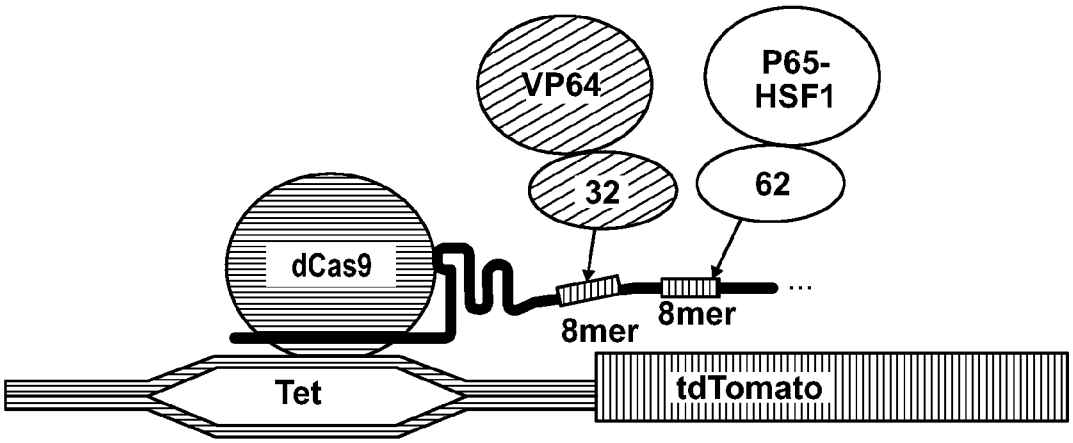

VP64
P65-HSF1
32
62
dCas9
8mer
8mer
...
Tet
tdTomato

FIG. 2B

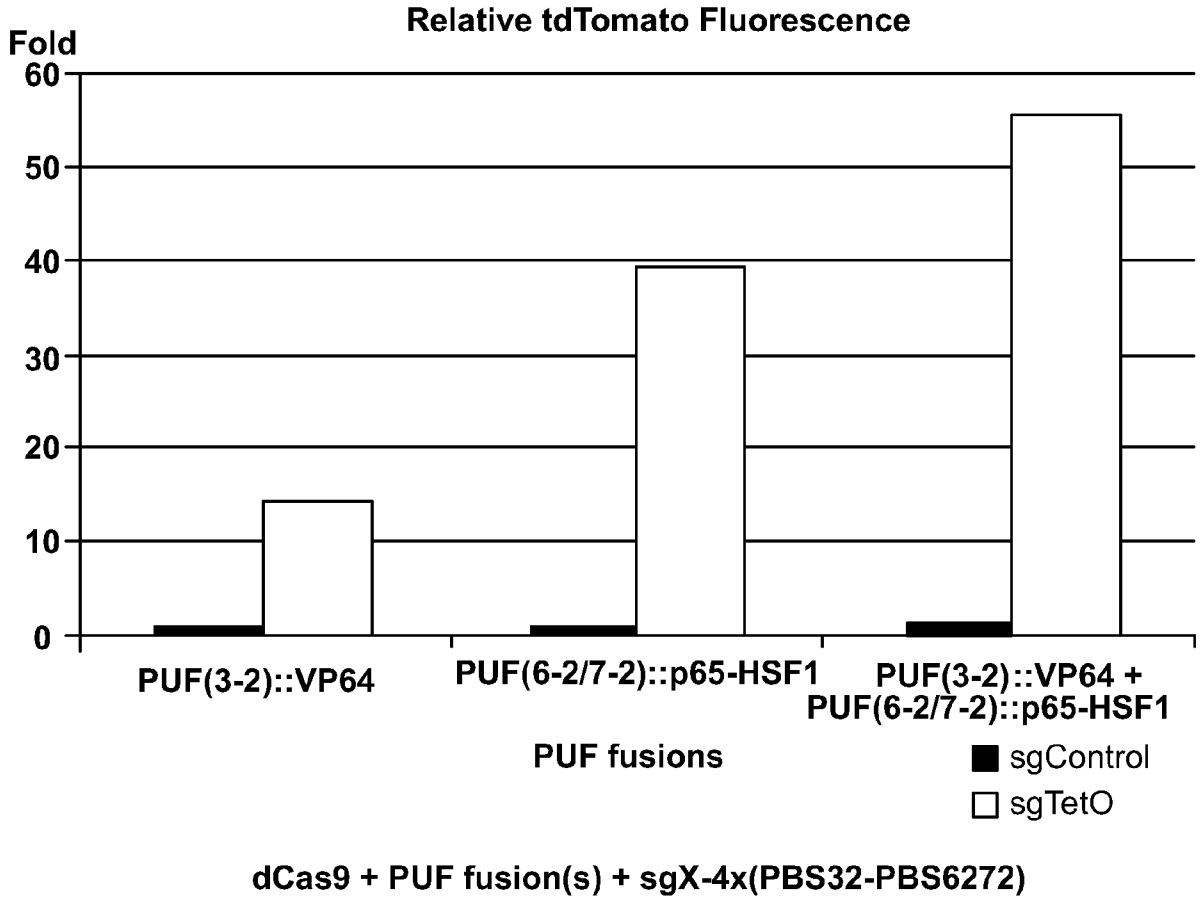
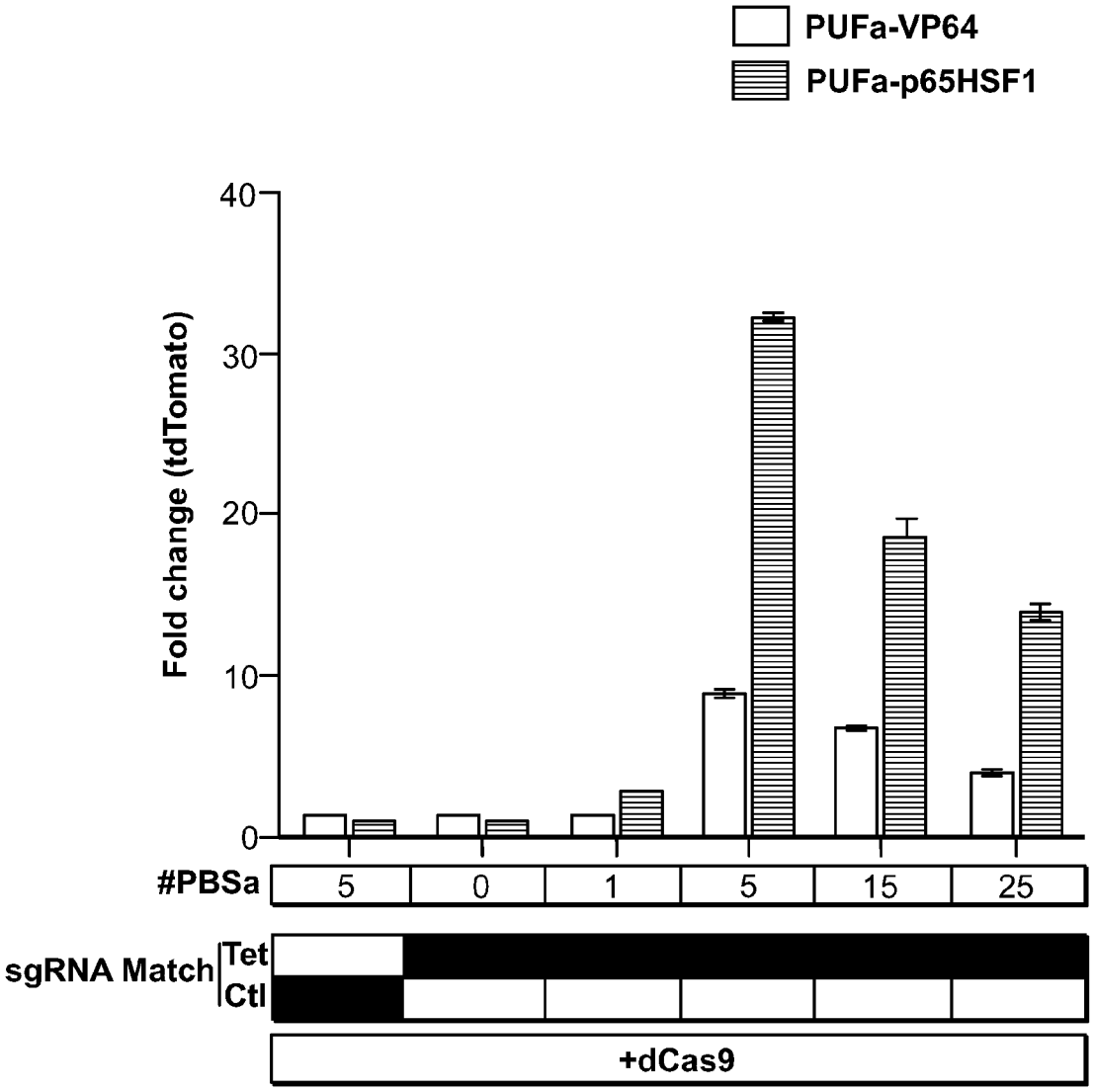

Relative tdTomato Fluorescence
Fold
60
50
40
30
20
10
0
PUF(3-2)::VP64
PUF(6-2/7-2)::p65-HSF1
PUF(3-2)::VP64 + PUF(6-2/7-2)::p65-HSF1
PUF fusions
sgControl
sgTetO
dCas9 + PUF fusion(s) + sgX-4x(PBS32-PBS6272)

FIG. 2C


PUFa-VP64
PUFa-p65HSF1
Fold change (tdTomato)
40
30
20
10
0
#PBSa
5
0
1
5
15
25
sgRNA Match
Tet
Ctl
+dCas9

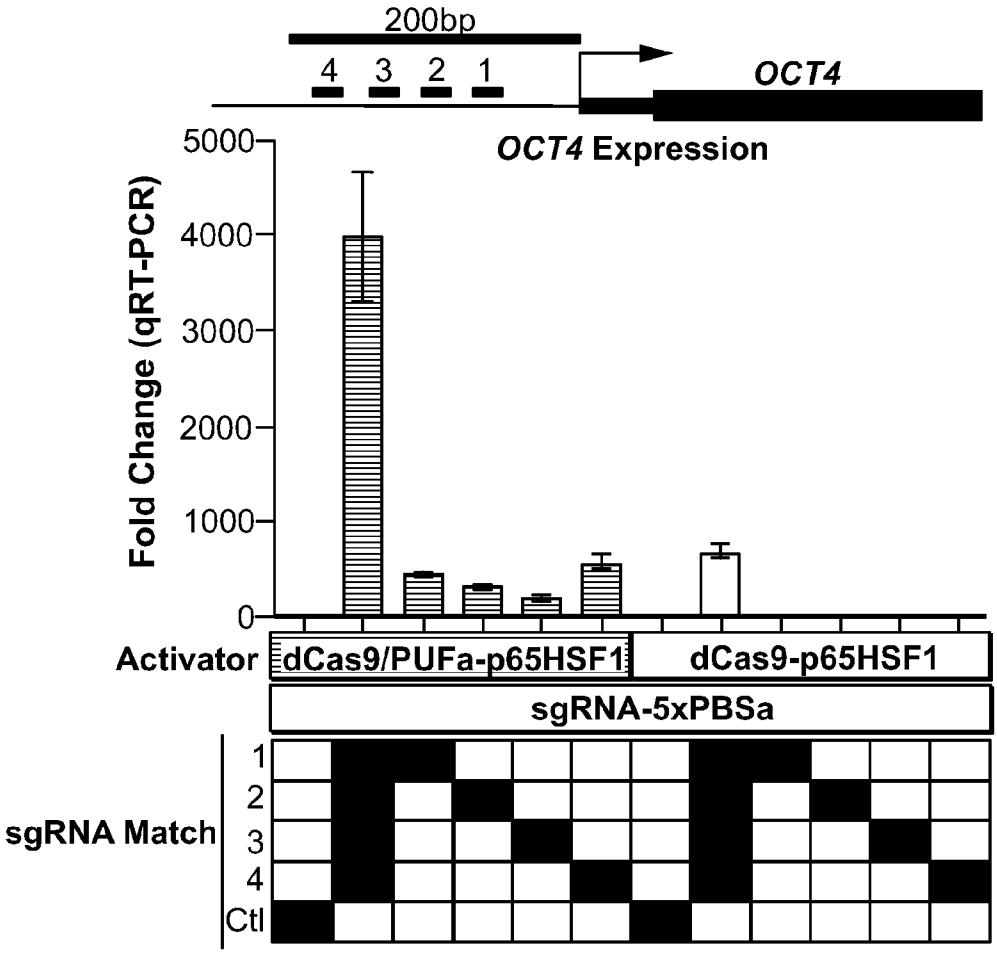

200bp
4 3 2 1
OCT4
OCT4 Expression
Fold Change (qRT-PCR)
5000
4000
3000
2000
1000
0
Activator
dCas9/PUFa-p65HSF1
dCas9-p65HSF1
sgRNA-5xPBSa
sgRNA Match
1
2
3
4
Ctl

FIG. 3A

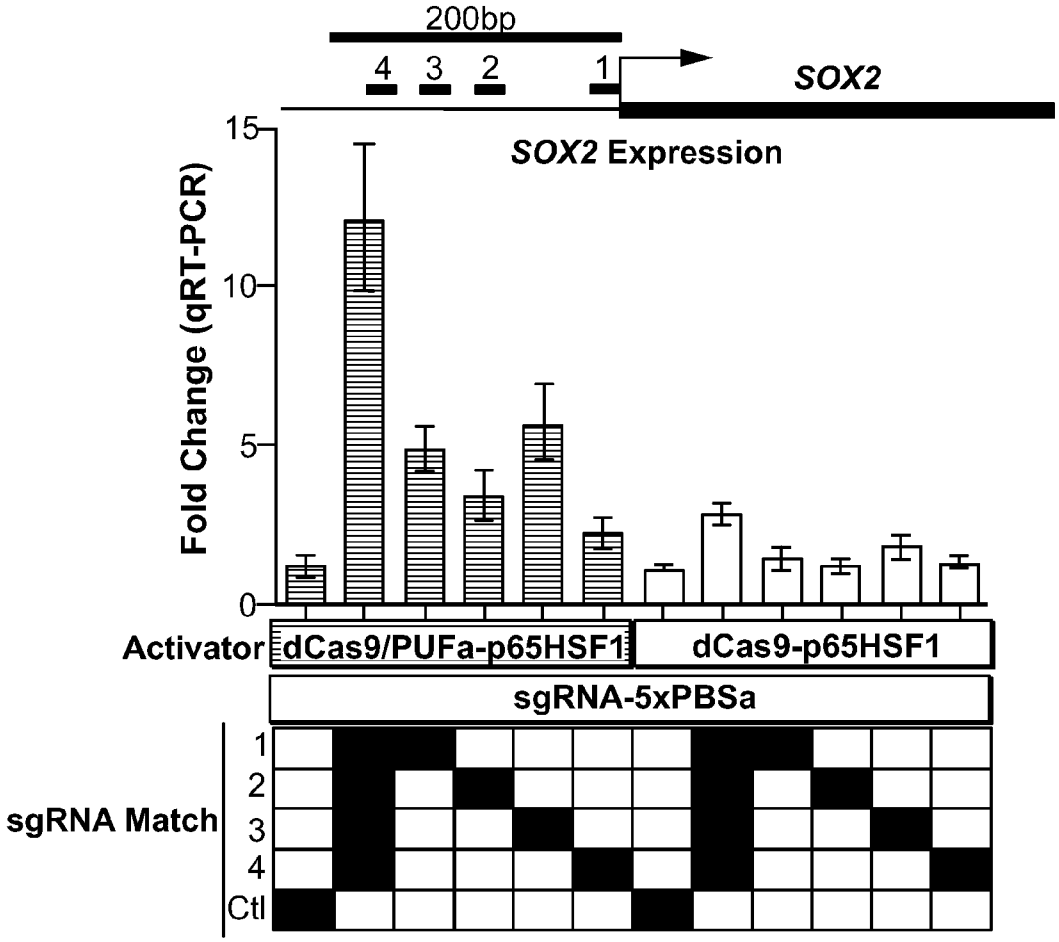

200bp
4 3 2 1
SOX2
SOX2 Expression
Fold Change (qRT-PCR)
15
10
5
0
Activator
dCas9/PUFa-p65HSF1
dCas9-p65HSF1
sgRNA-5xPBSa
sgRNA Match
1
2
3
4
Ctl

FIG. 3B

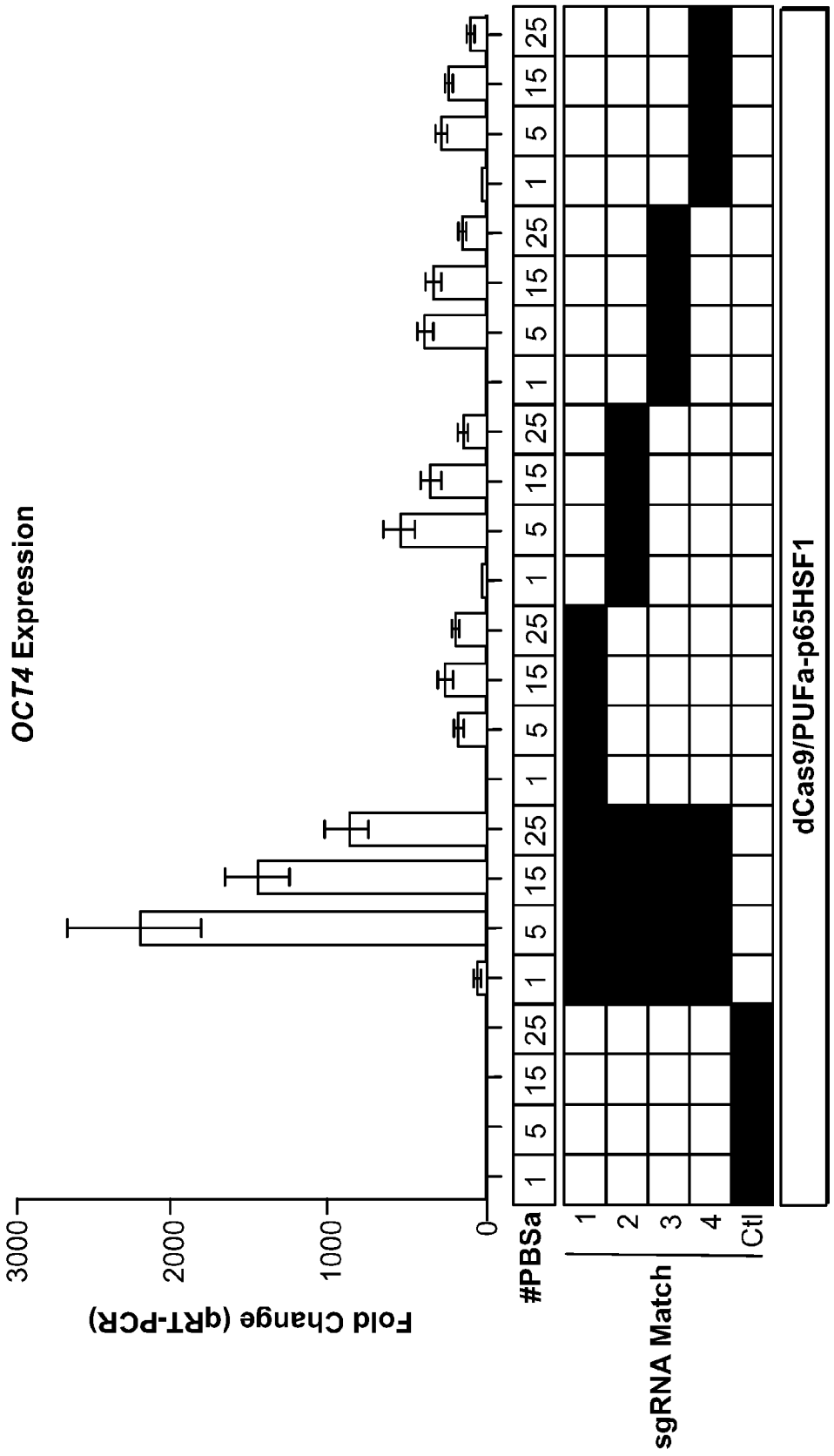

OCT4 Expression
Fold Change (qRT-PCR)
3000
2000
1000
0
#PBSa
1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25
sgRNA Match
1
2
3
4
Ctl
dCas9/PUFa-p65HSF1

FIG. 3C

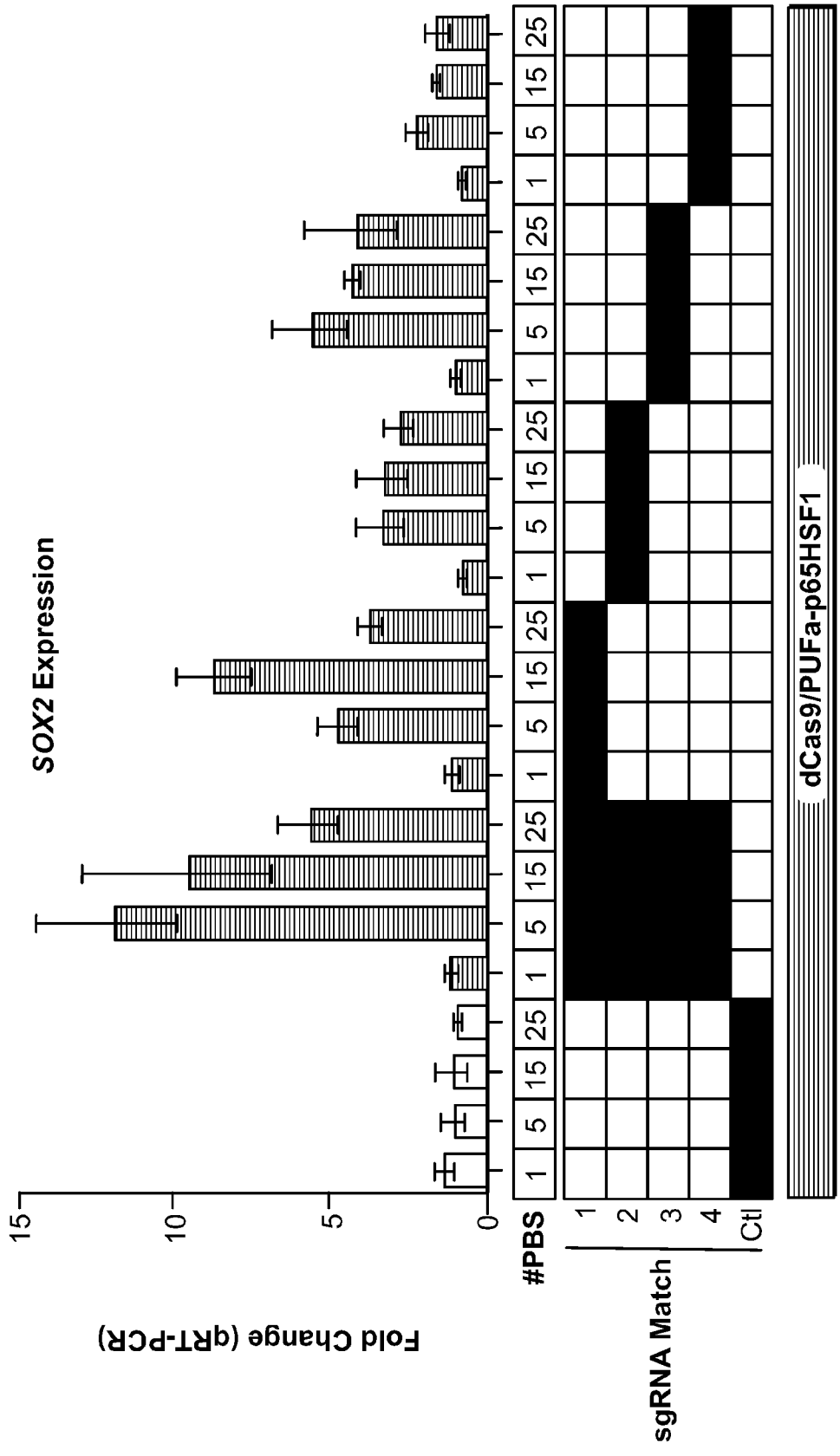

SOX2 Expression
Fold Change (qRT-PCR)
15
10
5
0
#PBS
1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25
sgRNA Match
1
2
3
4
Ctl
dCas9/PUFa-p65HSF1

FIG. 3D

FIG. 4A

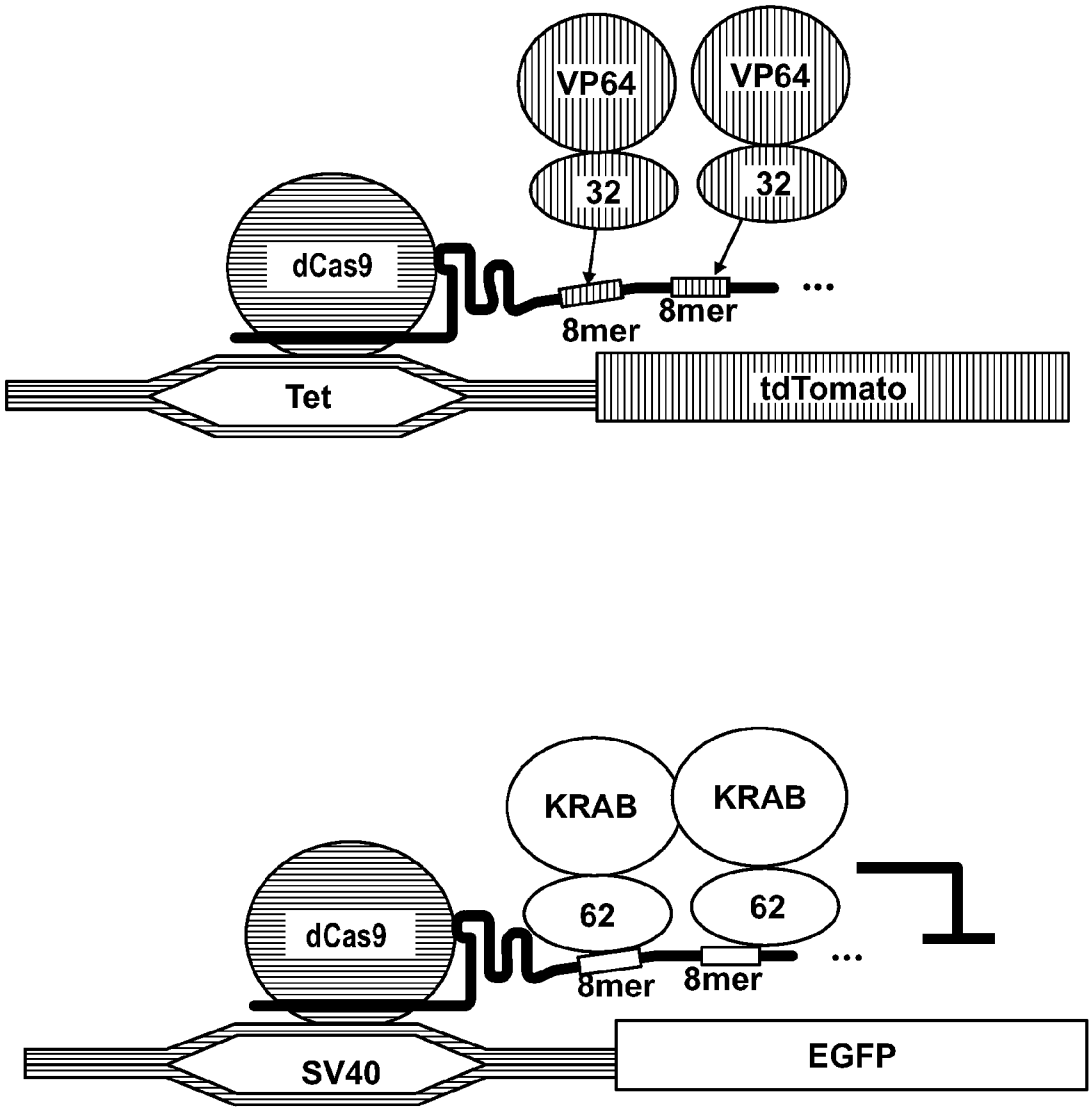
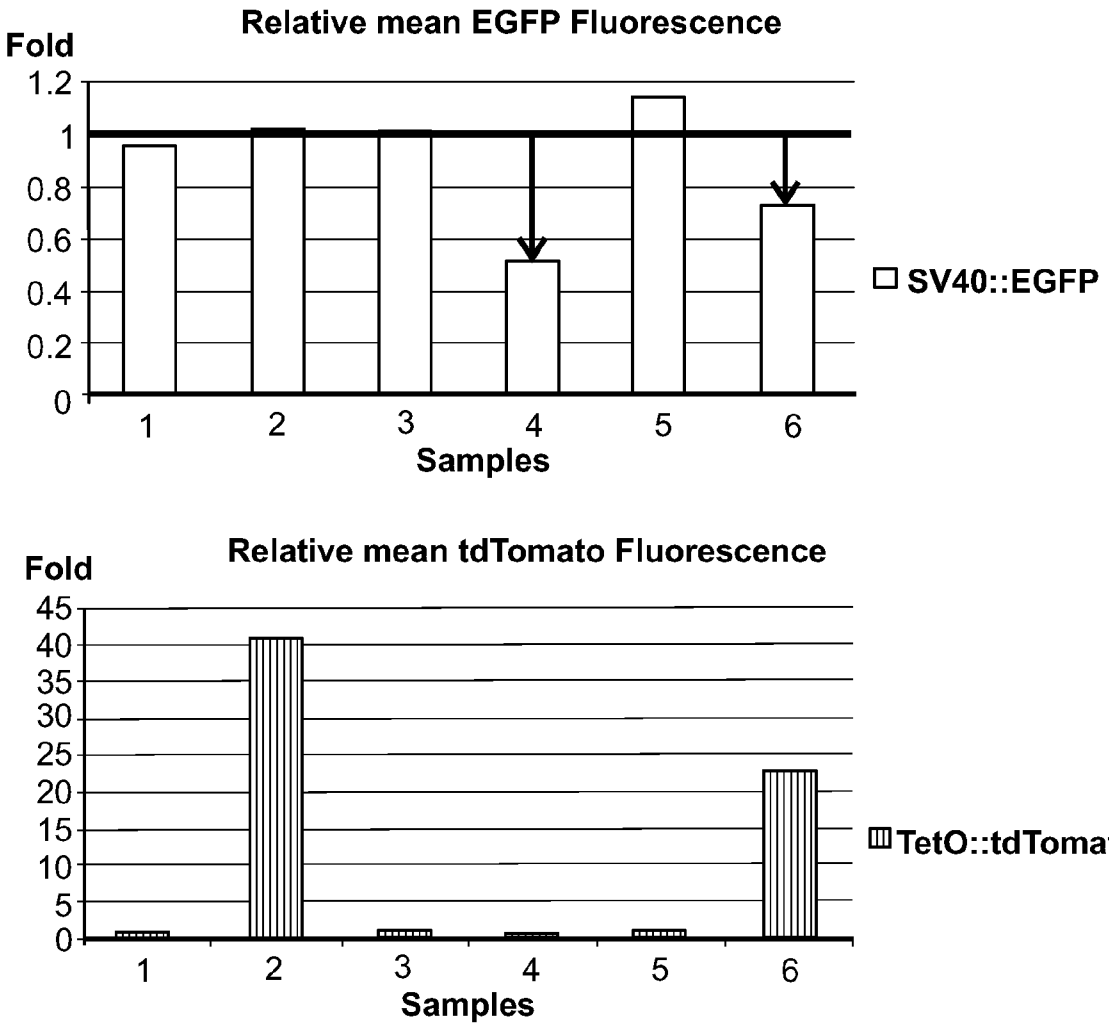

VP64
VP64
32
32
dCas9
8mer
8mer
...
Tet
tdTomato
KRAB
KRAB
62
62
dCas9
8mer
8mer
...
SV40
EGFP

FIG. 4B



	Samples
1	dCas9/sgControl-5xPBS32/PUF(3-2)::VP64
2	dCas9/sgTetO-5xPBS32/PUF(3-2)::VP64
3	dCas9/sgControl-5xPBS6272/KRAB::PUF(6-2/7-2)
4	dCas9/sgSV40-5xPBS6272/KRAB::PUF(6-2/7-2)
5	dCas9/sgControl-5xPBS32/PUF(3-2)::VP64/sgControl-5xPBS6272/KRAB::PUF(6-2/7-2)
6	dCas9/sgTetO-5xPBS32/PUF(3-2)::VP64/sgSV40-5xPBS6272/KRAB::PUF(6-2/7-2)

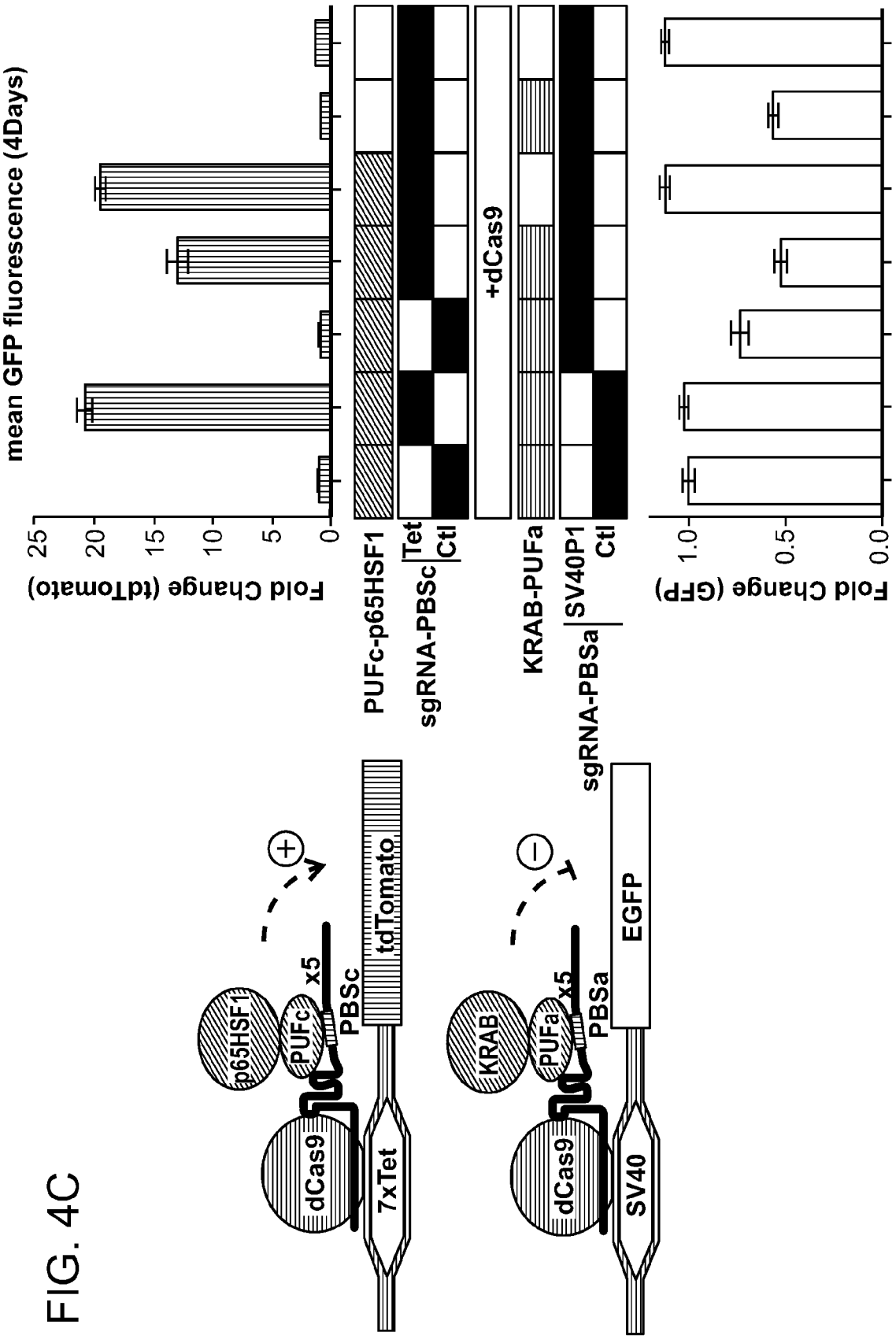

mean GFP fluorescence (4Days)
Fold Change (tdTomato)
25
20
15
10
5
0
PUFc-p65HSF1
sgRNA-PBSc
Tet
Ctl
+dCas9
KRAB-PUFa
sgRNA-PBSa
SV40P1
Ctl
Fold Change (GFP)
1.0
0.5
0.0
p65HSF1
PUFc
x5
PBSc
tdTomato
dCas9
7xTet
KRAB
PUFa
x5
PBSa
EGFP
dCas9
SV40

FIG. 4C

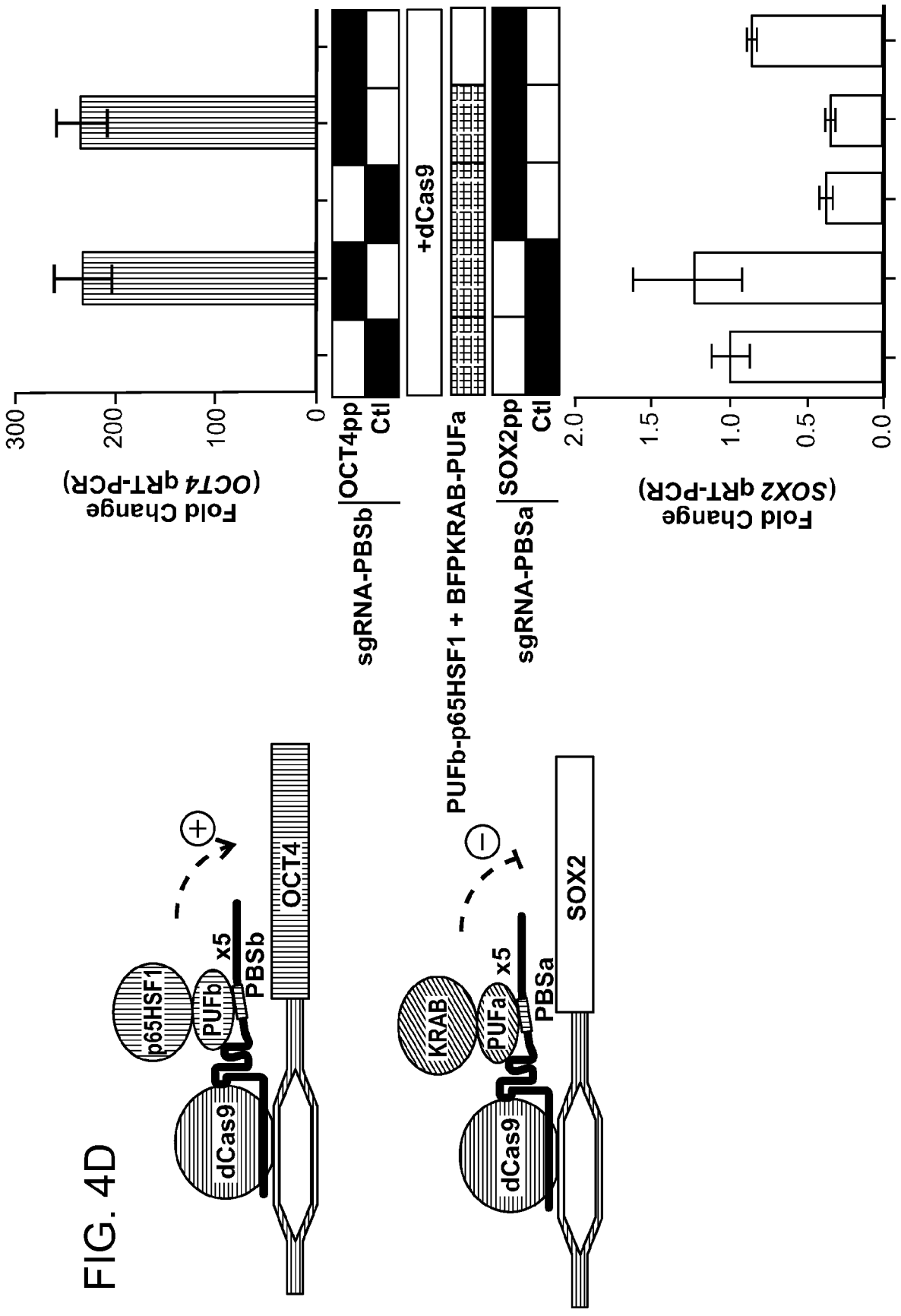

Fold Change
(OCT4 qRT-PCR)
300
200
100
0
OCT4pp
Ctl
sgRNA-PBSb
+dCas9
PUFb-p65HSF1 + BFPKRAB-PUFa
SOX2pp
Ctl
sgRNA-PBSa
Fold Change
(SOX2 qRT-PCR)
2.0
1.5
1.0
0.5
0.0
p65HSF1
PUFb
x5
PBSb
OCT4
dCas9
KRAB
PUFa
x5
PBSa
SOX2
dCas9

FIG. 4D

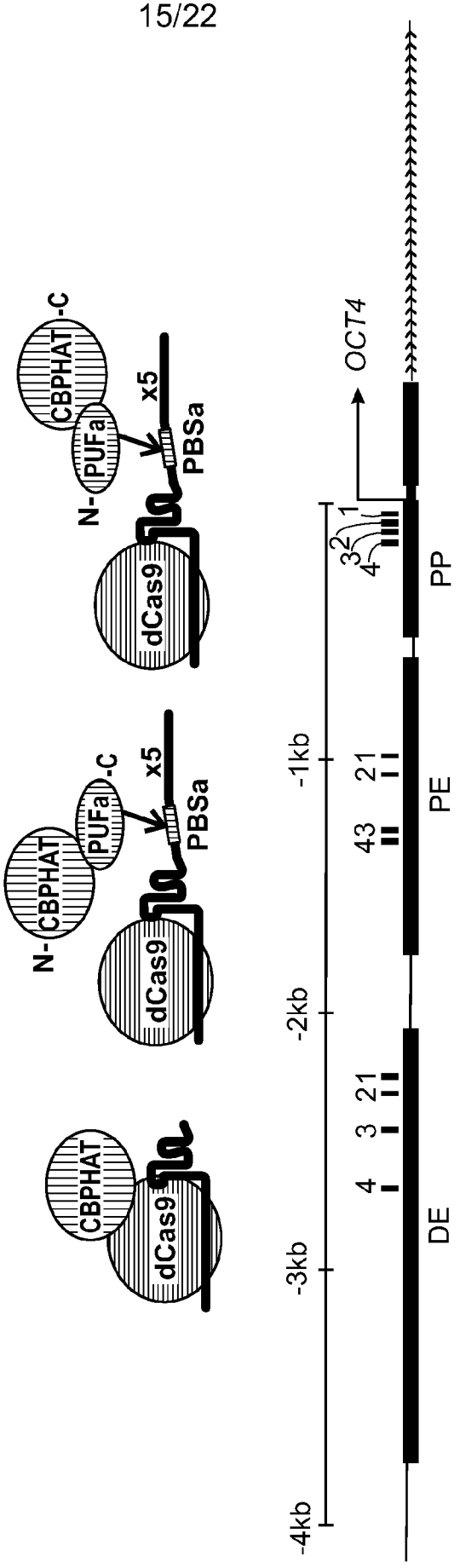

CBPHAT
dCas9
N-
CBPHAT
PUFa
-C
x5
PBSa
dCas9
N-
CBPHAT
-C
PUFa
x5
PBSa
dCas9
OCT4
-1kb
-2kb
-3kb
-4kb
4
3
21
DE
43
21
PE
3
2
1
4
PP

FIG. 5A

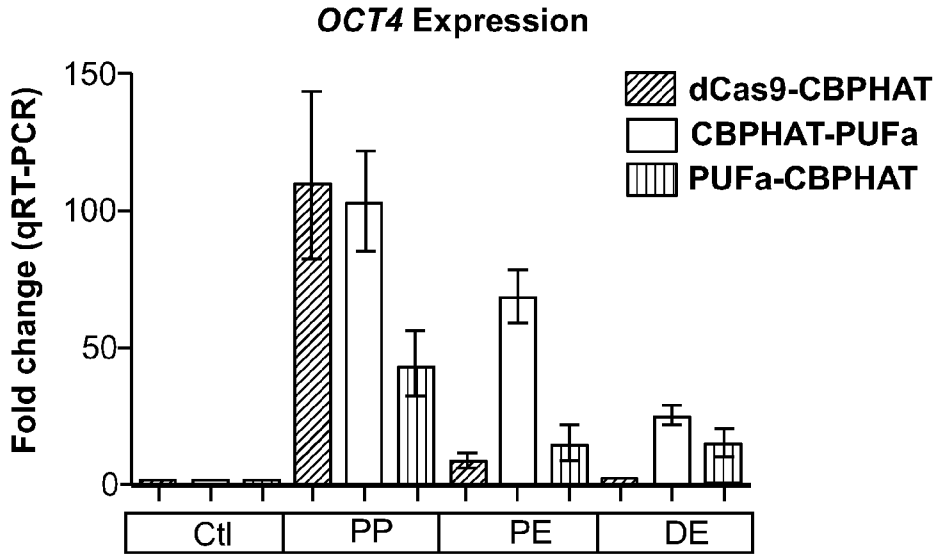

OCT4 Expression
Fold change (qRT-PCR)
150
100
50
0
dCas9-CBPHAT
CBPHAT-PUFa
PUFa-CBPHAT
Ctl
PP
PE
DE

FIG. 5B

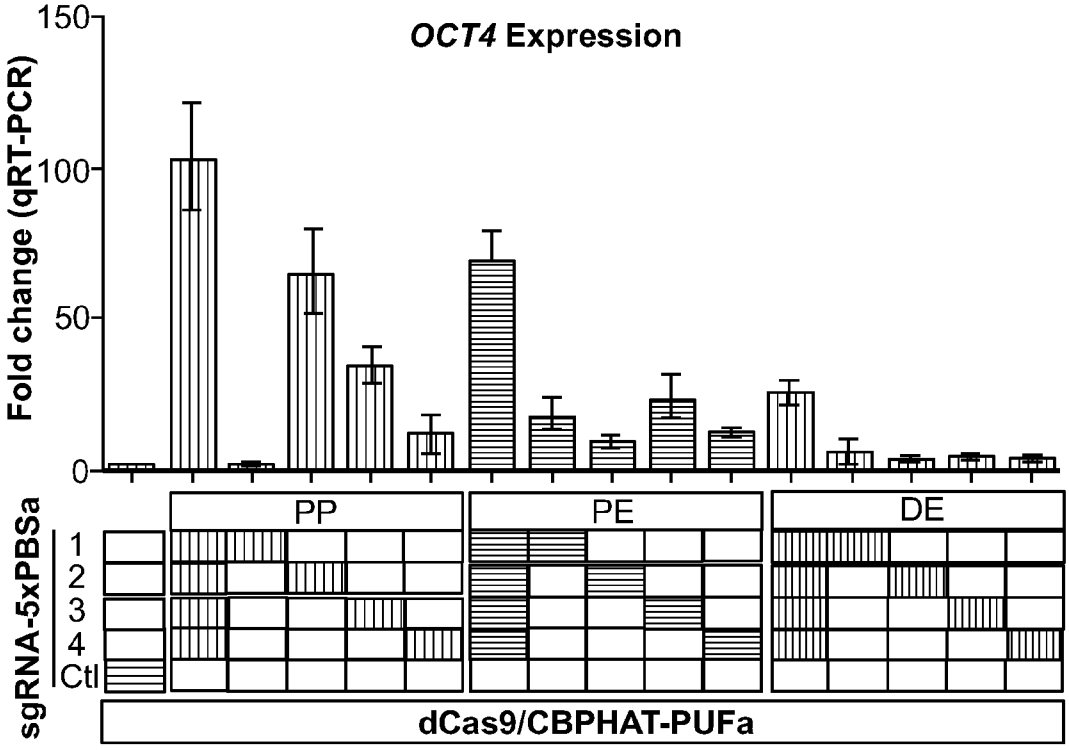

OCT4 Expression
Fold change (qRT-PCR)
150
100
50
0
PP
PE
DE
sgRNA-5xPBSa
1
2
3
4
Ctl
dCas9/CBPHAT-PUFa

FIG. 5C

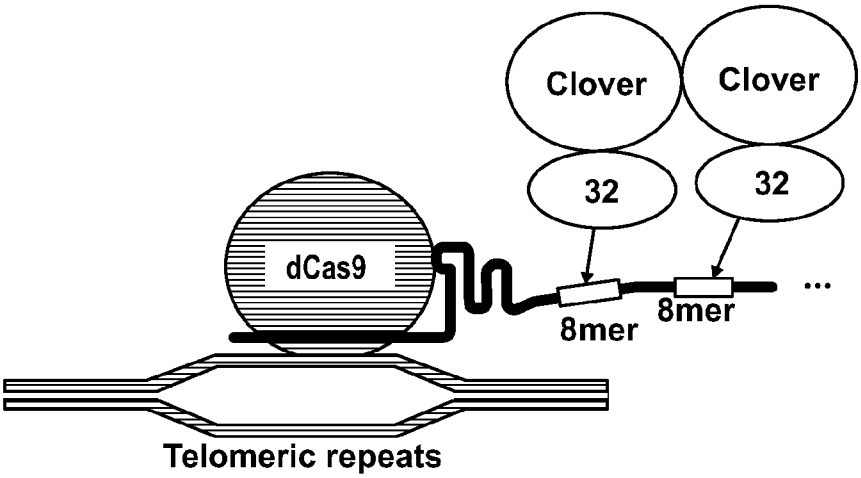


FIG. 6A

FIG. 6B

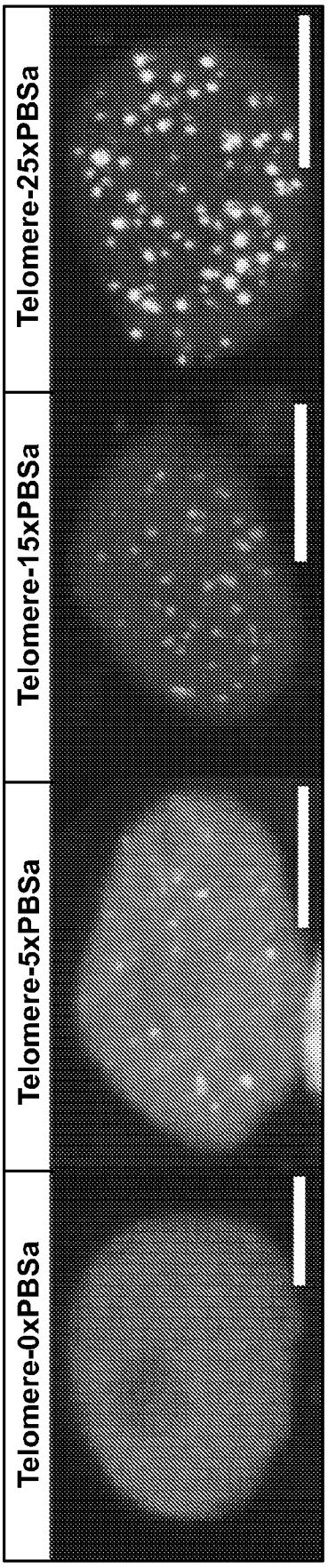

Telomere-0xPBSa
Telomere-5xPBSa
Telomere-15xPBSa
Telomere-25xPBSa

FIG. 6C

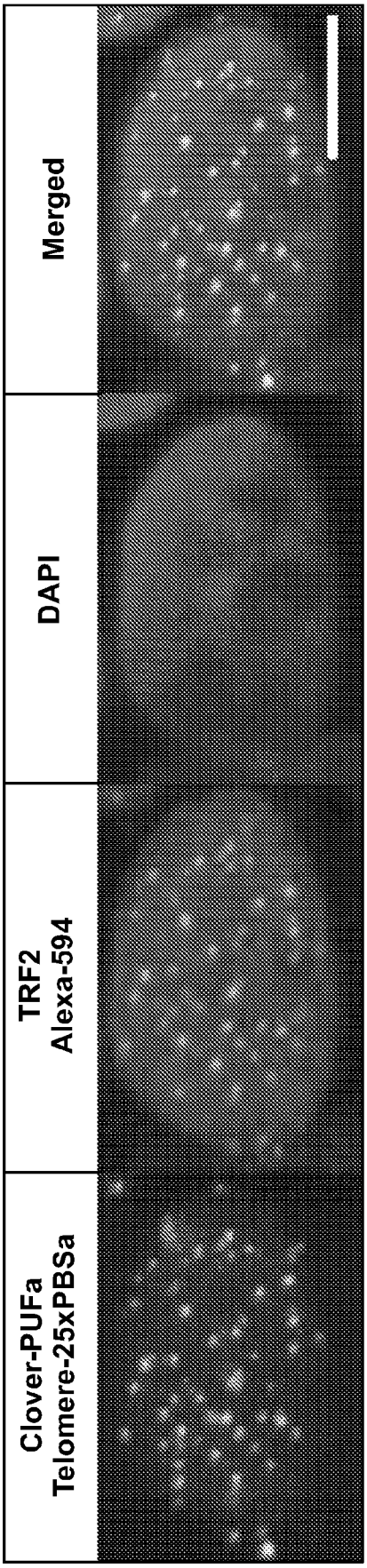

Clover-PUFa
Telomere-25xPBSa
TRF2
Alexa-594
DAPI
Merged

FIG. 6D

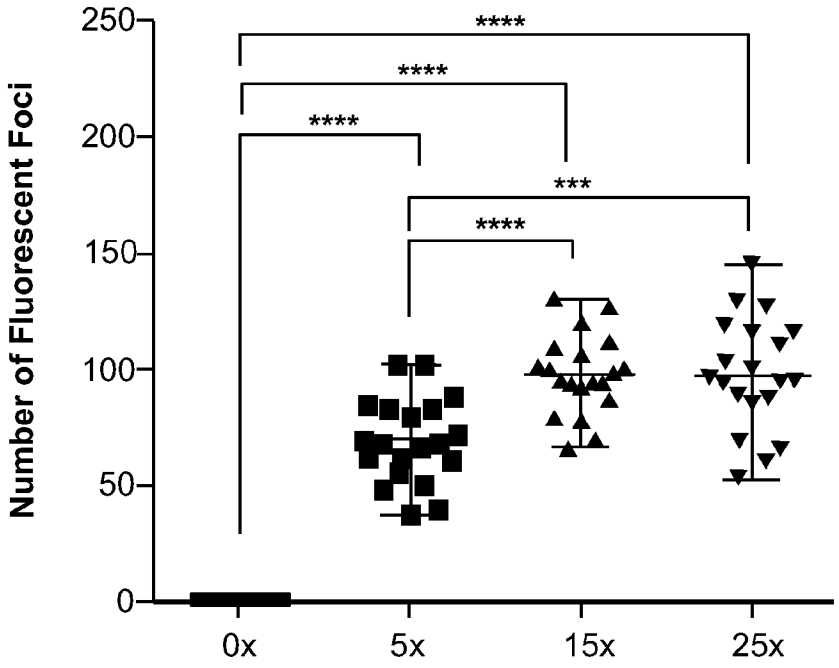

Number of Fluorescent Foci
250
200
150
100
50
0
****
****
****
***
****
0x
5x
15x
25x

FIG. 6E

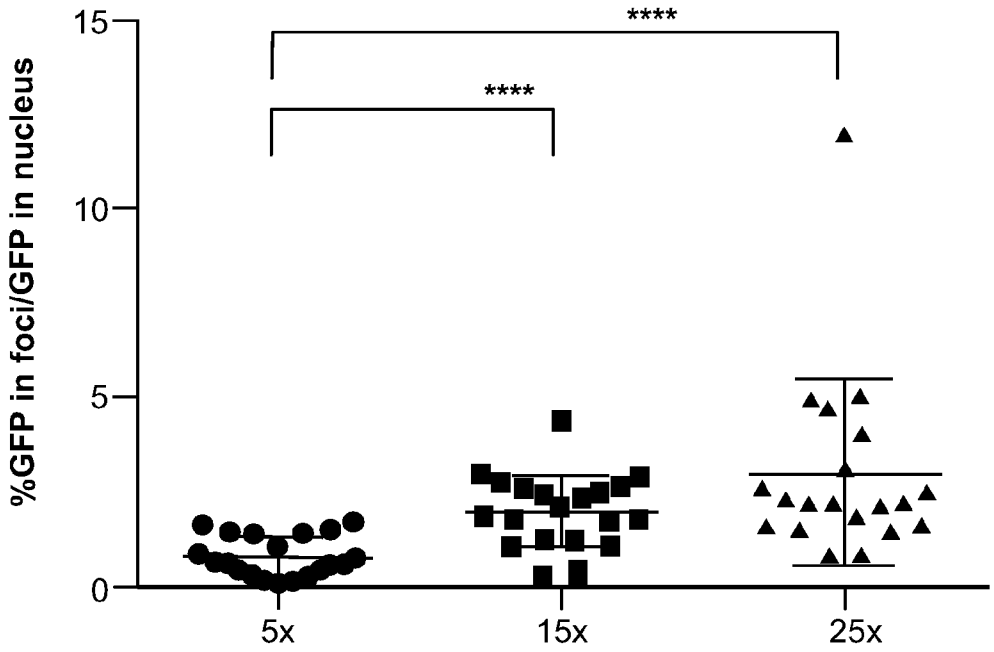

%GFP in foci/GFP in nucleus
15
10
5
0
****
****
5x
15x
25x

FIG. 6F

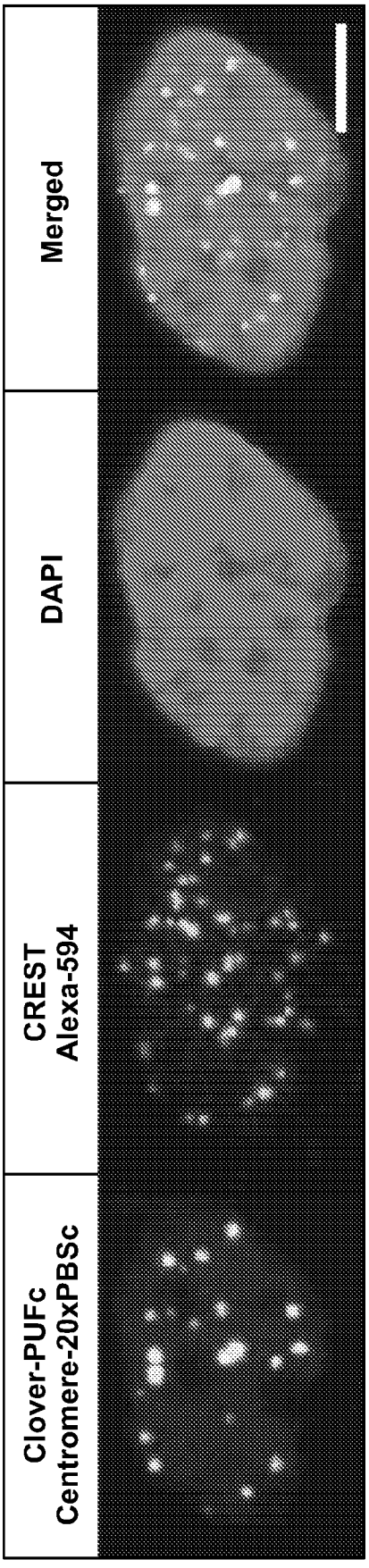
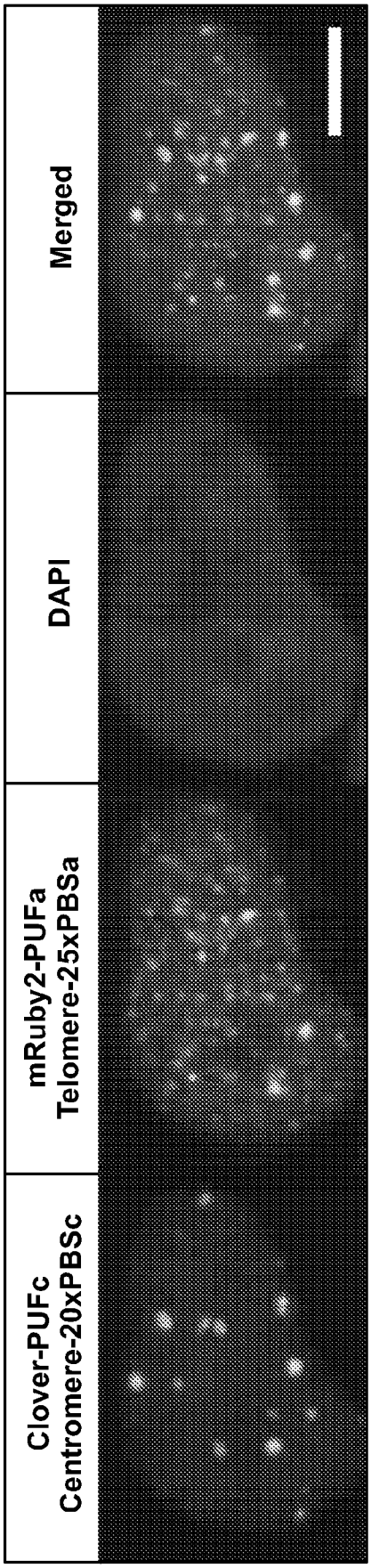

Clover-PUFc
Centromere-20xPBSc
CREST
Alexa-594
DAPI
Merged

FIG. 6G


Clover-PUFc
Centromere-20xPBSc
mRuby2-PUFa
Telomere-25xPBSa
DAPI
Merged

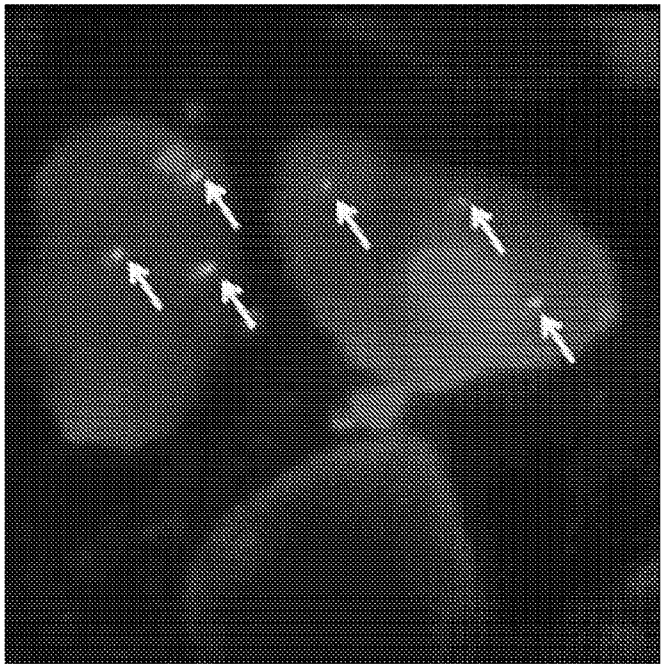


FIG. 7

FIG. 8A

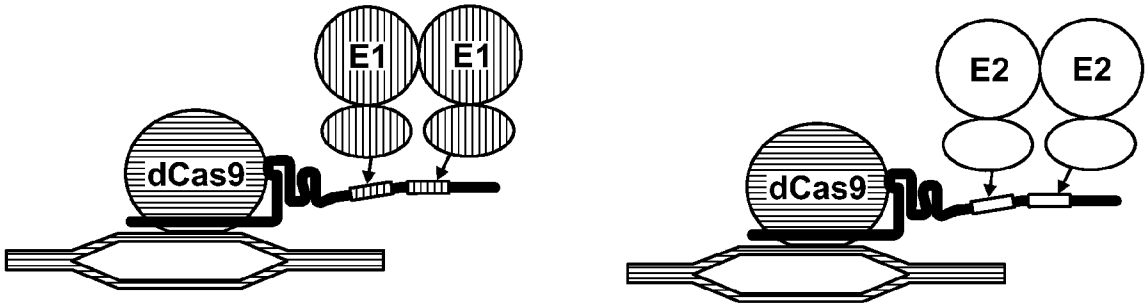


FIG. 8B

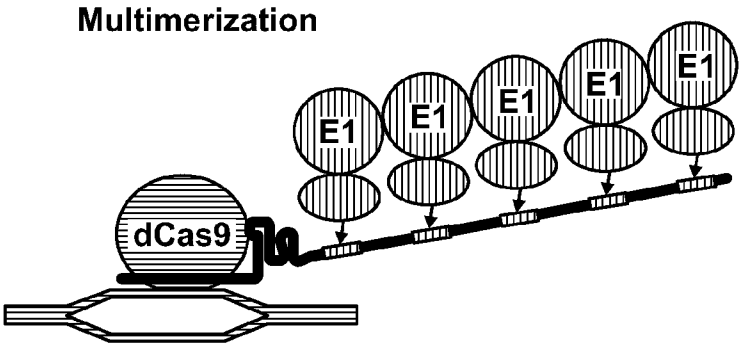


FIG. 8C

