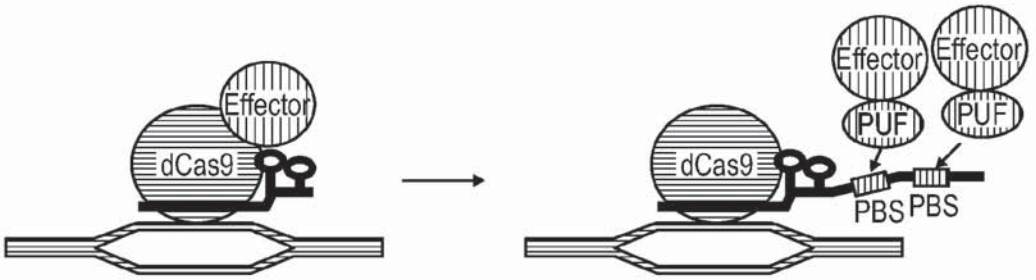
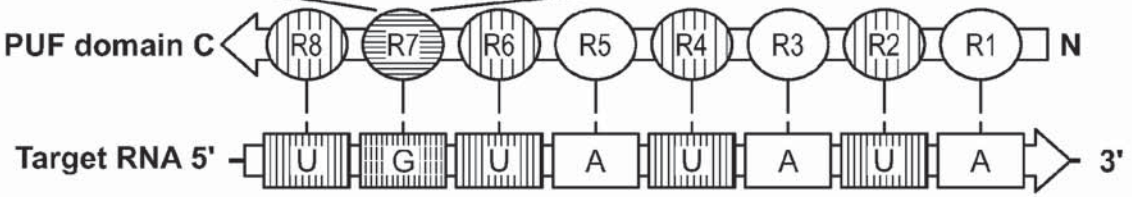
FIG. 1A

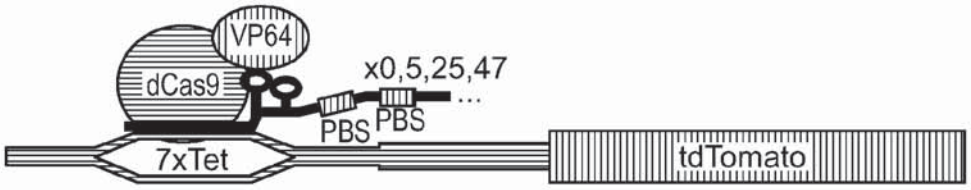


Example diresidue	Base
Gln Cys	A
Gln Asn	U
Glu Ser	G
Arg Ser	C



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. 1B


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

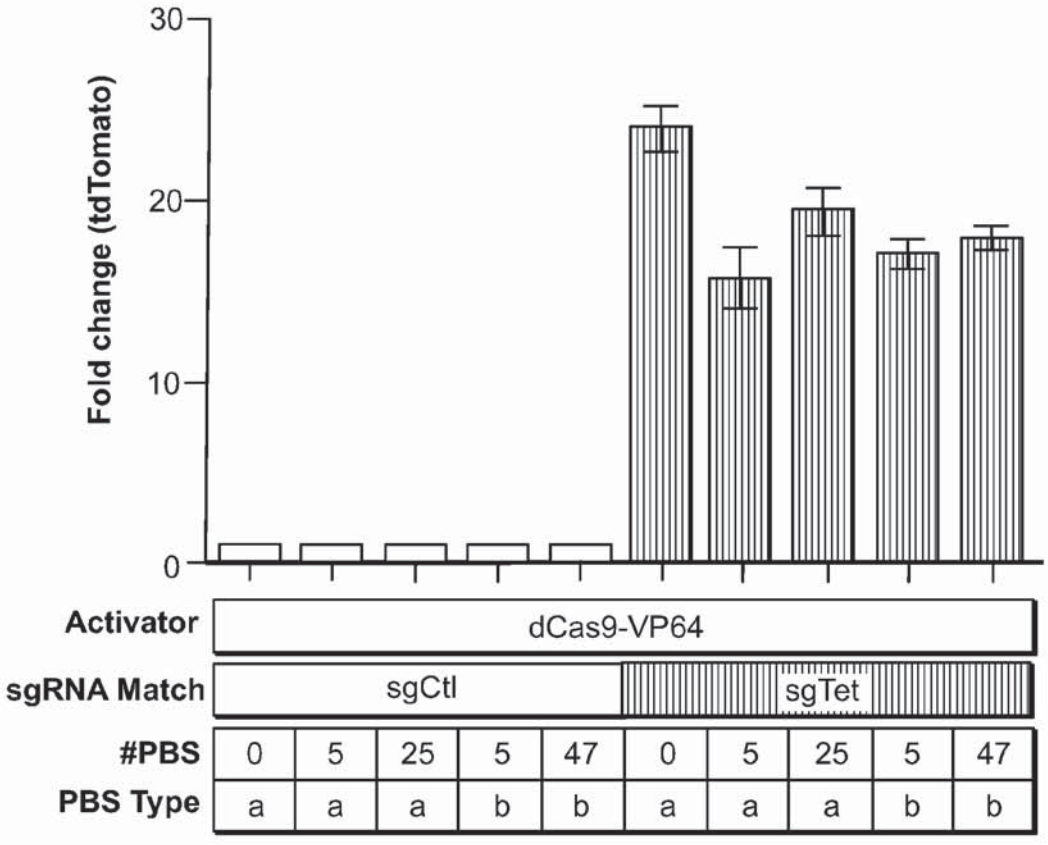
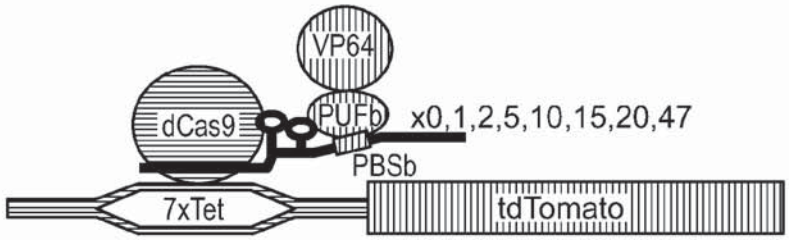

Fold change (tdTomato)
30
20
10
0
Activator
dCas9-VP64
sgRNA Match
sgCtl
sgTet
#PBS
0 5 25 5 47 0 5 25 5 47
PBS Type
a a a b b a a a b b

FIG. 1C


VP64
dCas9
PUFb
x0,1,2,5,10,15,20,47
PBSb
7xTet
tdTomato

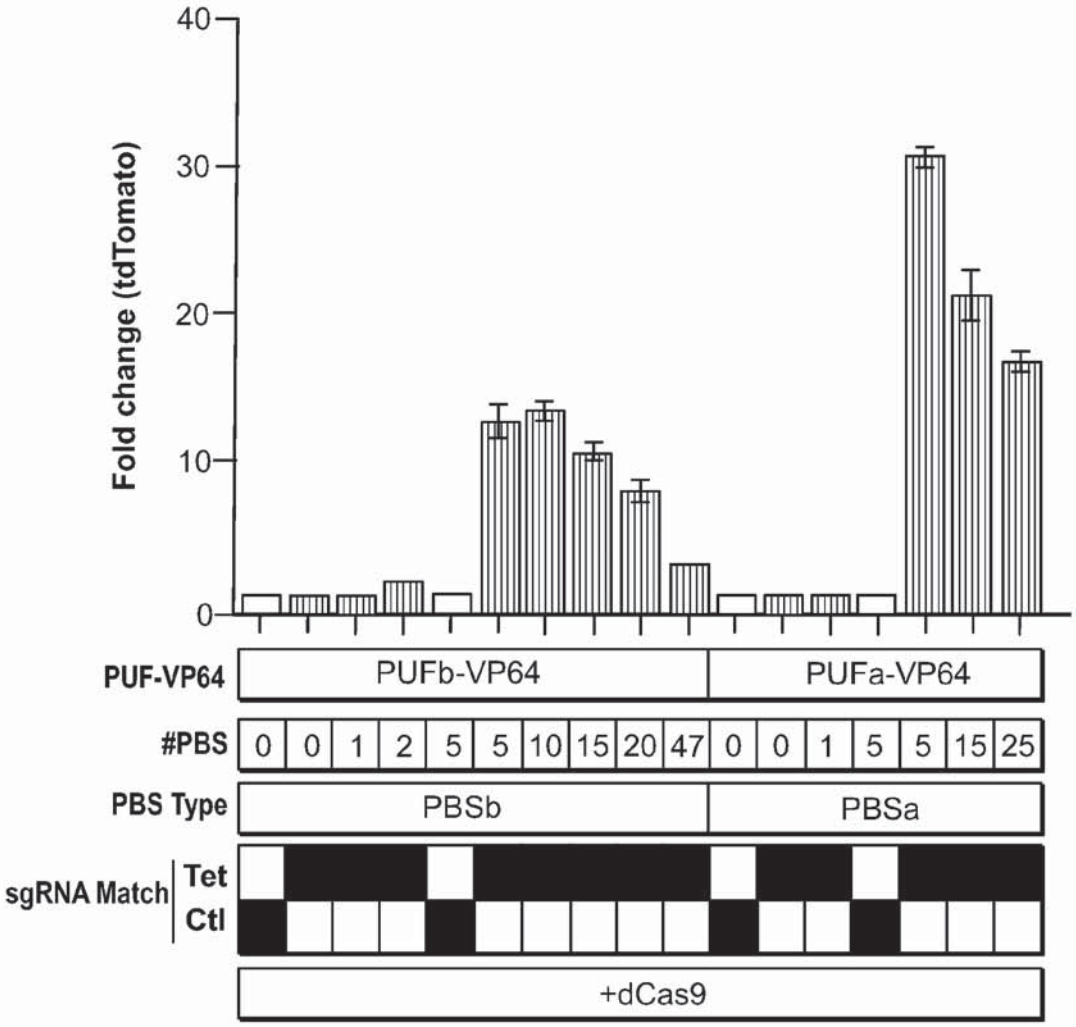

Fold change (tdTomato)
40
30
20
10
0
PUF-VP64
PUFb-VP64
PUFa-VP64
#PBS
0 0 1 2 5 5 10 15 20 47 0 0 1 5 5 15 25
PBS Type
PBSb
PBSa
sgRNA Match
Tet
Ctl
+dCas9

FIG. 1D

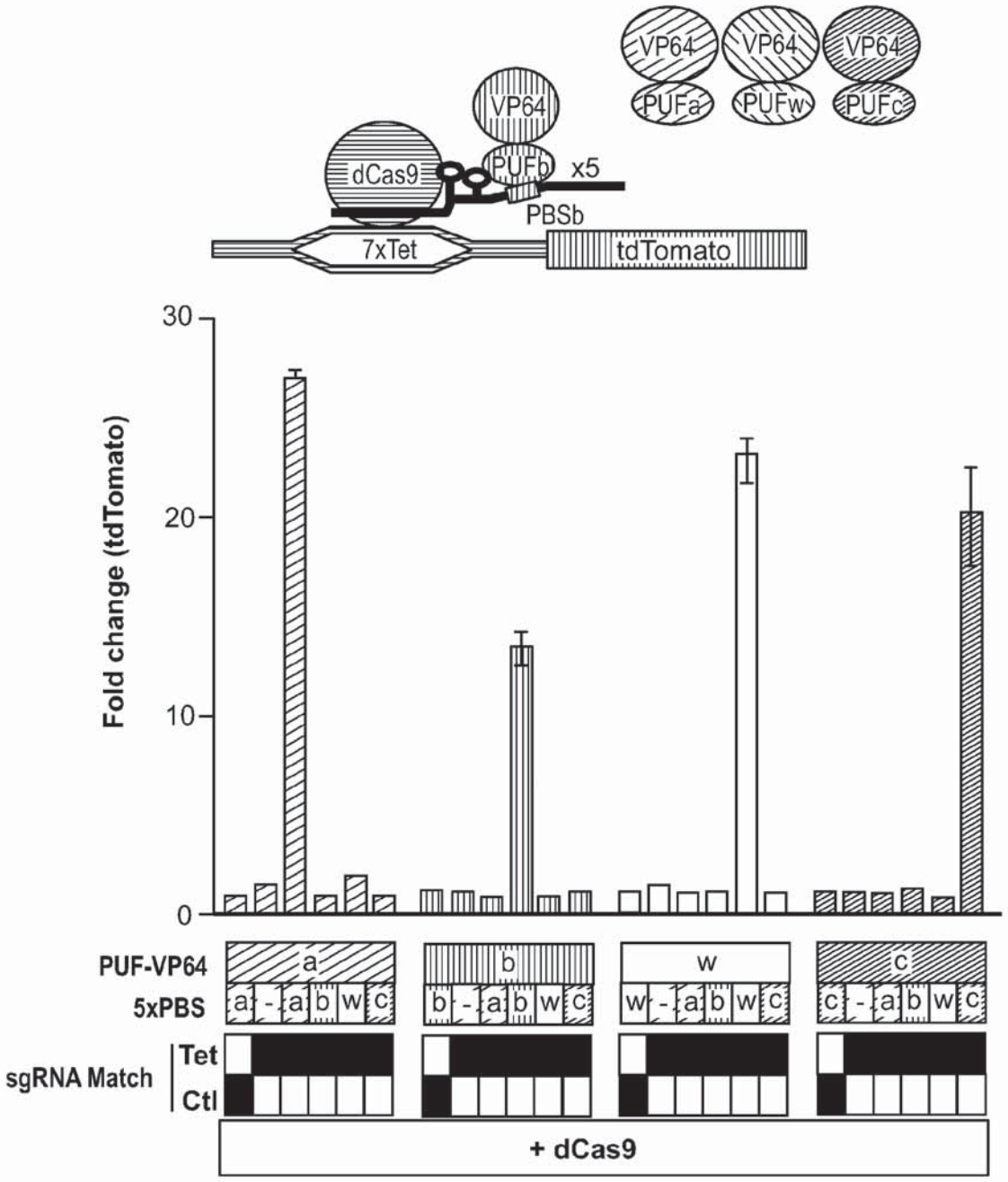

VP64
VP64
VP64
PUFa
PUFw
PUFc
VP64
dCas9
PUFb
x5
PBSb
7xTet
tdTomato
30
20
10
0
Fold change (tdTomato)
PUF-VP64
5xPBS
a
b
w
c
a - a b w c
b - a b w c
w - a b w c
c - a b w c
sgRNA Match
Tet
Ctl
+ dCas9

FIG. 2A

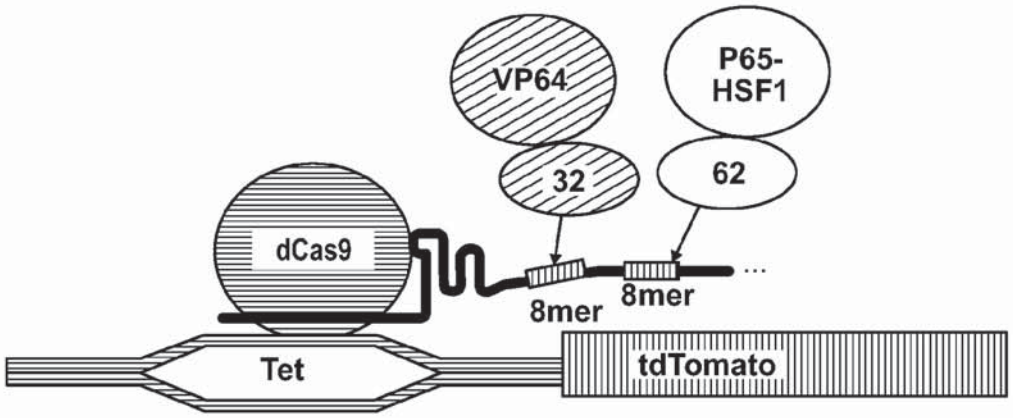

VP64
P65-
HSF1
32
62
dCas9
8mer
8mer
...
Tet
tdTomato

FIG. 2B

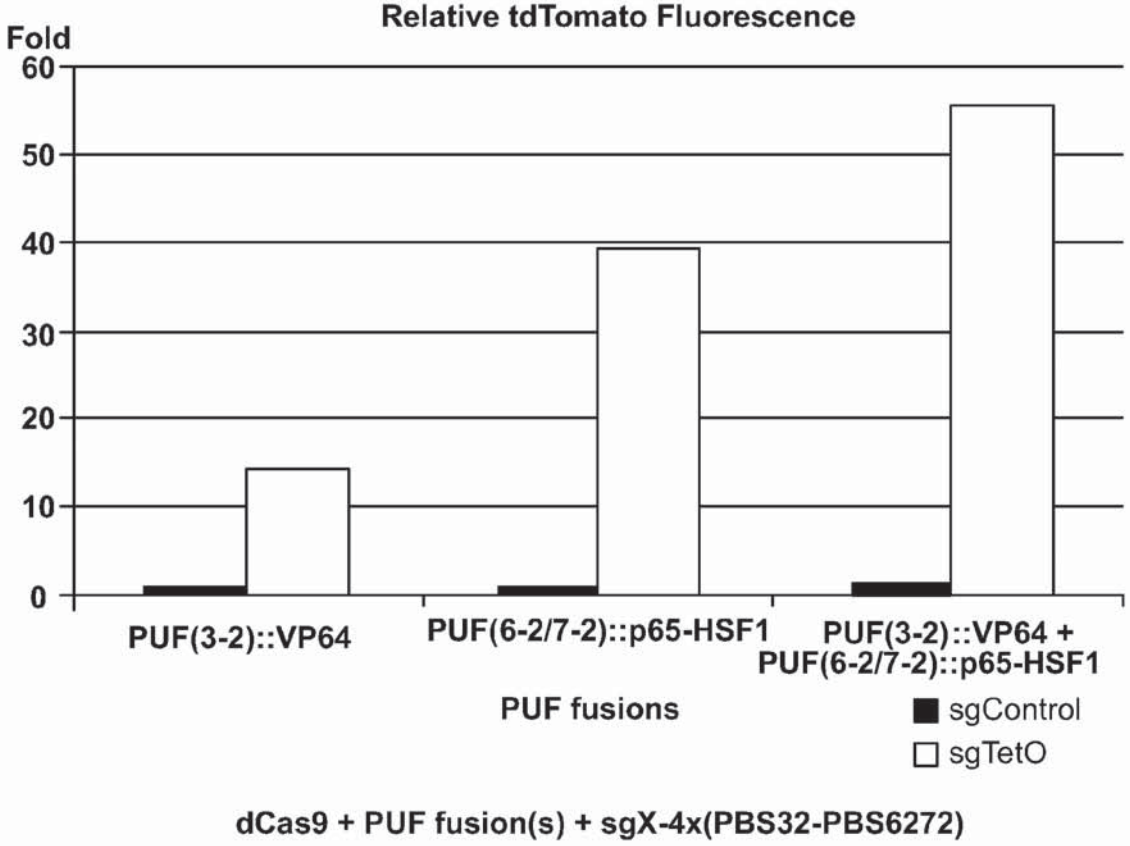
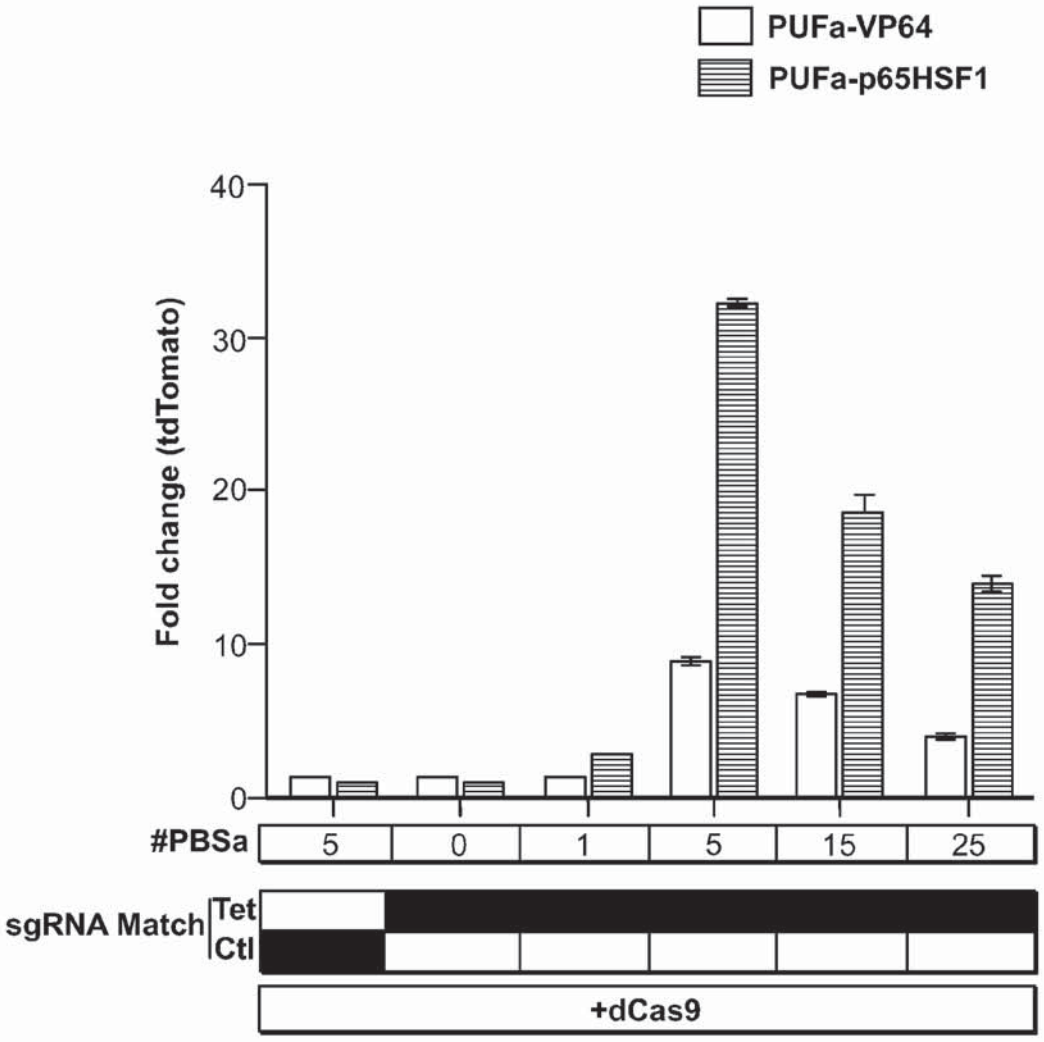

Relative tdTomato Fluorescence
Fold
60
50
40
30
20
10
0
PUF(3-2)::VP64
PUF(6-2/7-2)::p65-HSF1
PUF(3-2)::VP64 +
PUF(6-2/7-2)::p65-HSF1
PUF fusions
sgControl
sgTetO
dCas9 + PUF fusion(s) + sgX-4x(PBS32-PBS6272)

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

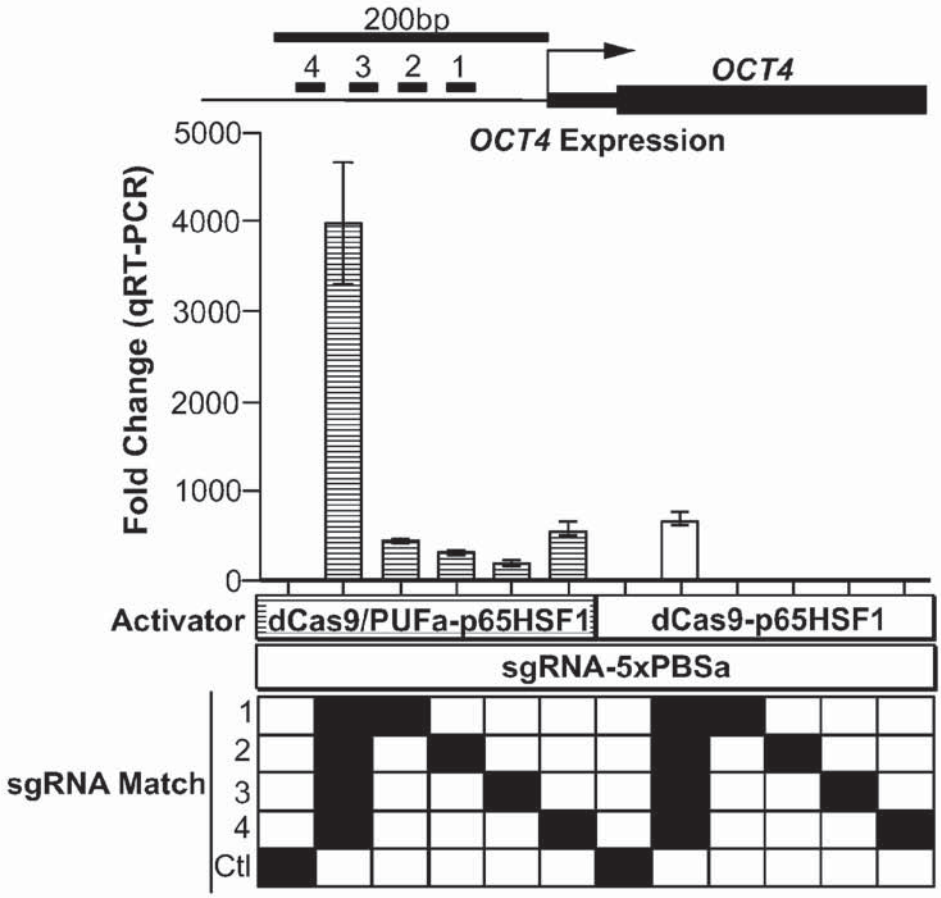

200bp
4 3 2 1
OCT4
OCT4 Expression
Fold Change (qRT-PCR)
5000
4000
3000
2000
1000
0
Activator
dCas9/PUFa-p65HSF1
dCas9-p65HSF1
sgRNA-5xPBSa
sgRNA Match
1
2
3
4
Ctl

FIG. 3A

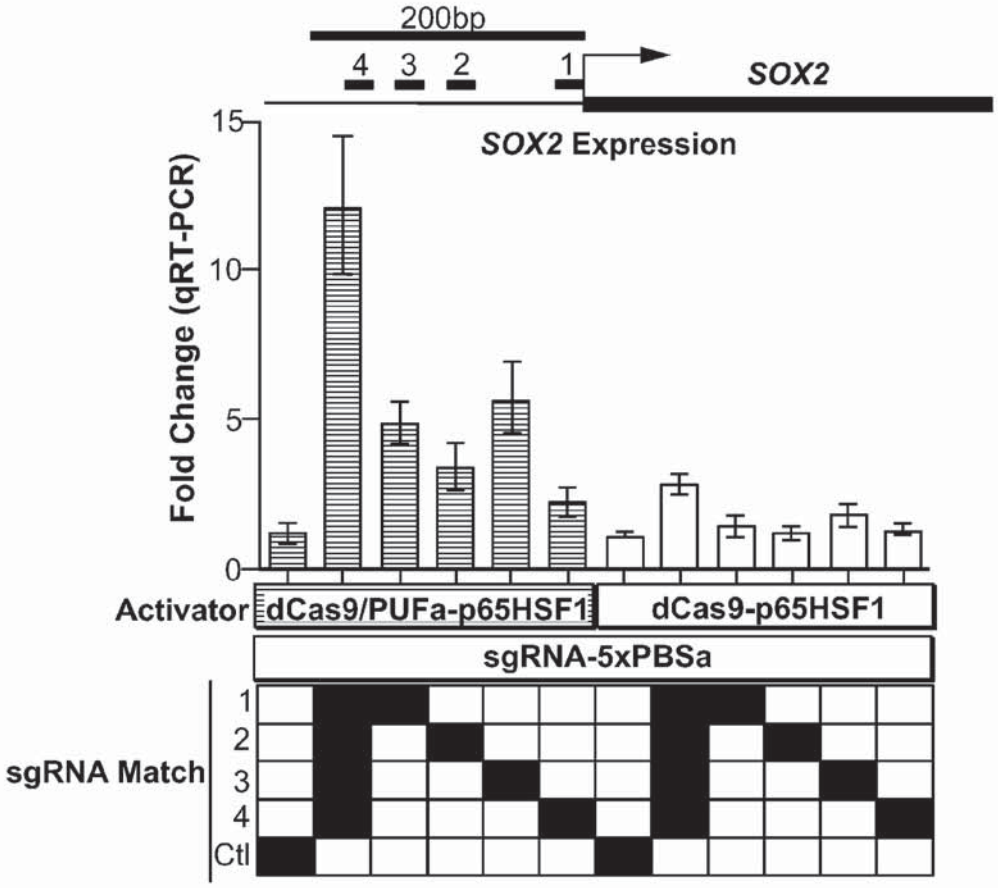

200bp
4
3
2
1
SOX2
SOX2 Expression
Fold Change (qRT-PCR)
15
10
5
0
Activator
dCas9/PUFa-p65HSF1
dCas9-p65HSF1
sgRNA-5xPBSa
sgRNA Match
1
2
3
4
Ctl

FIG. 3B

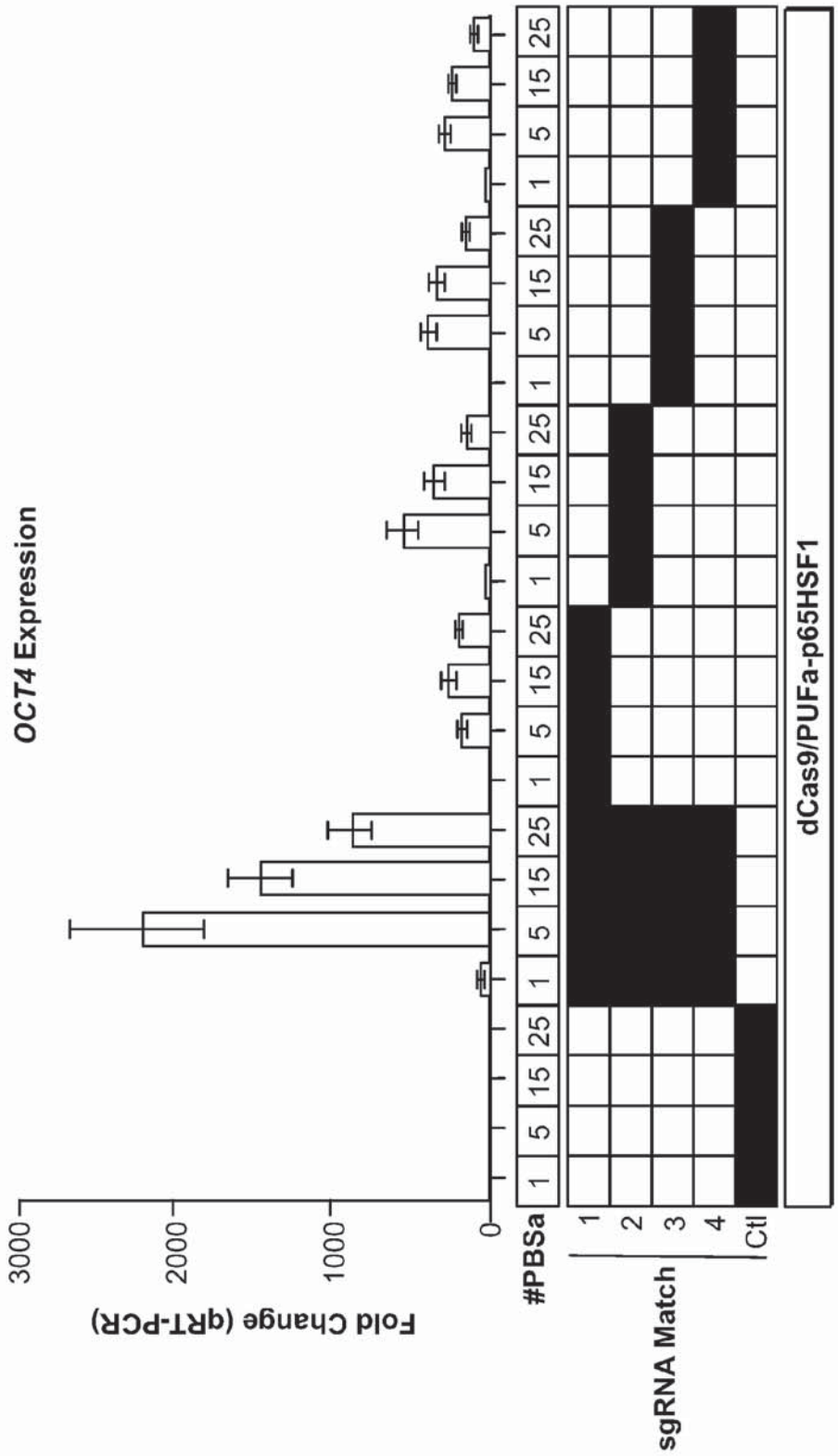

OCT4 Expression
Fold Change (qRT-PCR)
3000
2000
1000
0
#PBSa
1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25 1 5 15 25
sgRNA Match
1
2
3
4
Ctl
dCas9/PUFa-p65HSF1

FIG. 3C

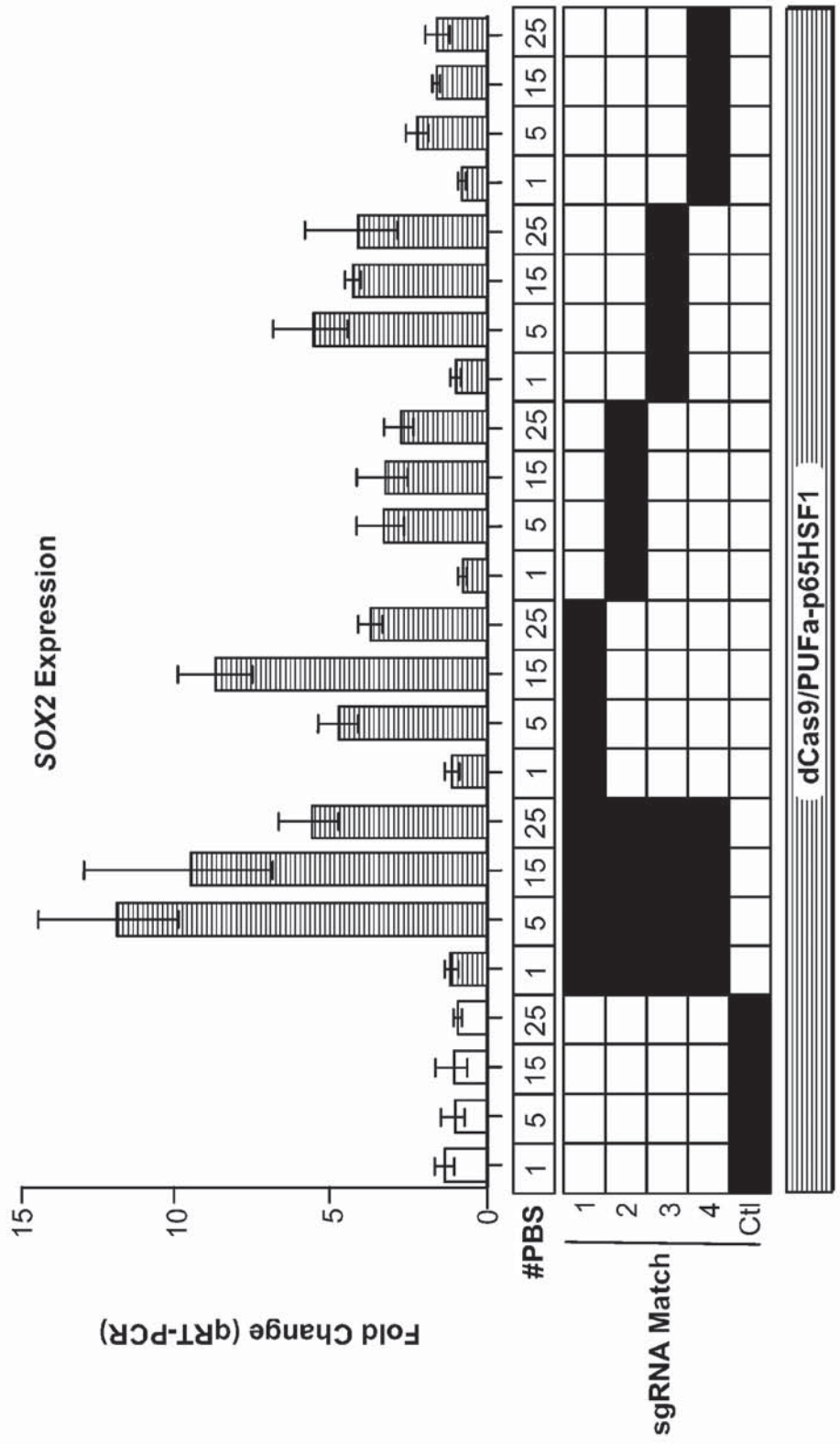

SOX2 Expression
Fold Change (qRT-PCR)
15
10
5
0
#PBS
1
5
15
25
1
5
15
25
1
5
15
25
1
5
15
25
sgRNA Match
1
2
3
4
Ctl
dCas9/PUFa-p65HSF1

FIG. 3D

FIG. 4A

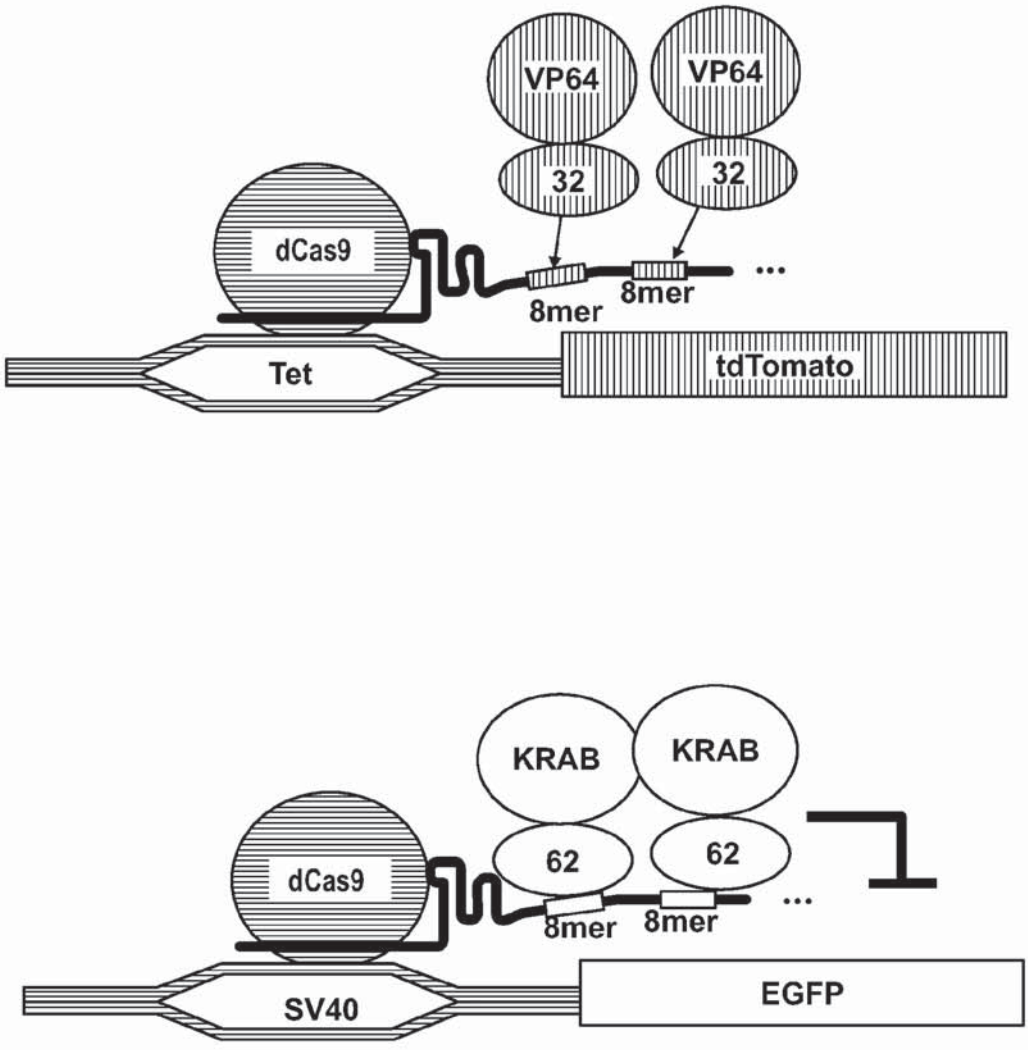
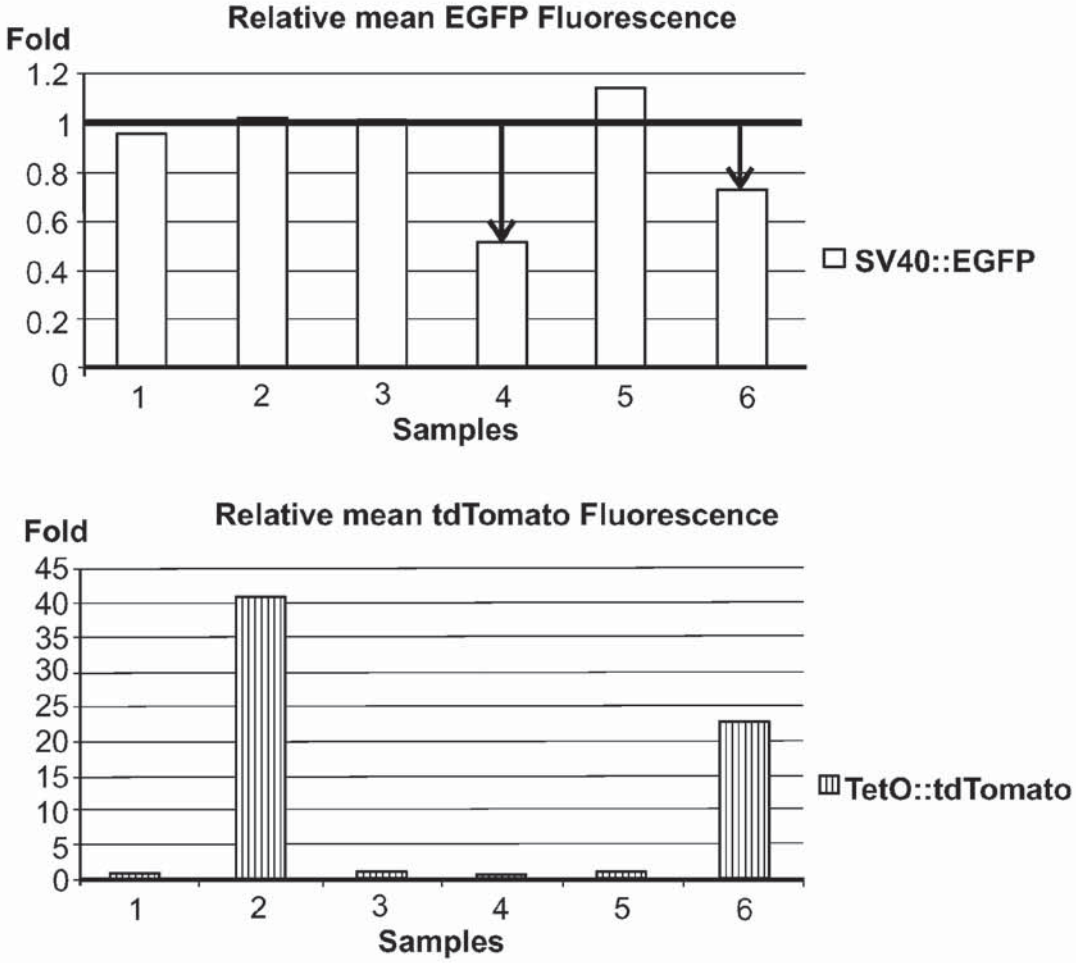

VP64
VP64
32
32
dCas9
8mer
8mer
...
Tet
tdTomato
KRAB
KRAB
62
62
dCas9
8mer
8mer
...
SV40
EGFP

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)

FIG. 4C

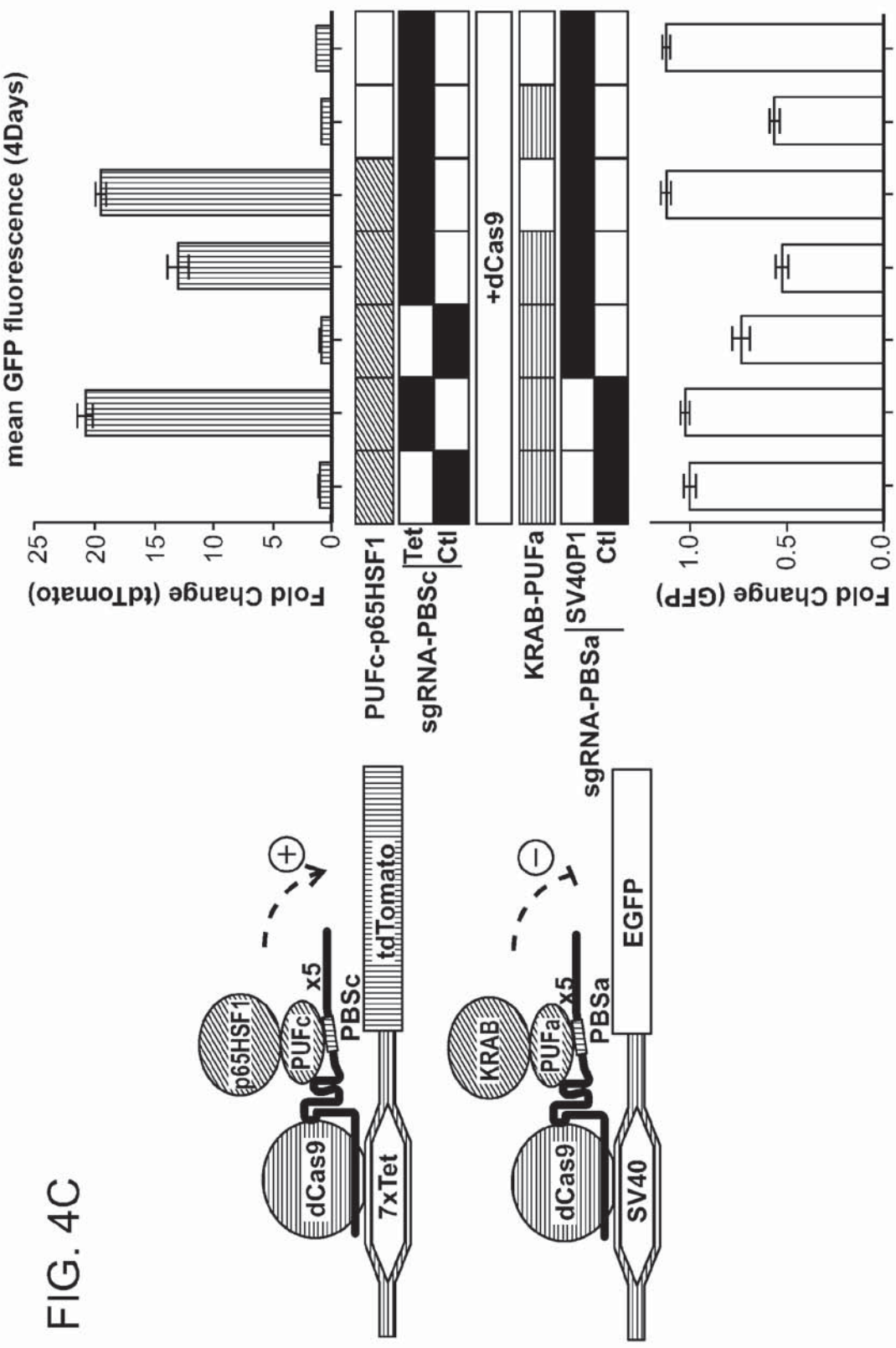
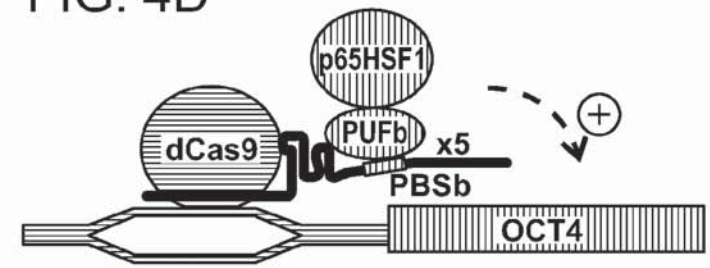
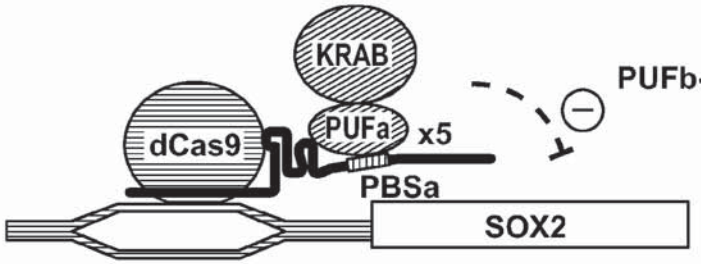
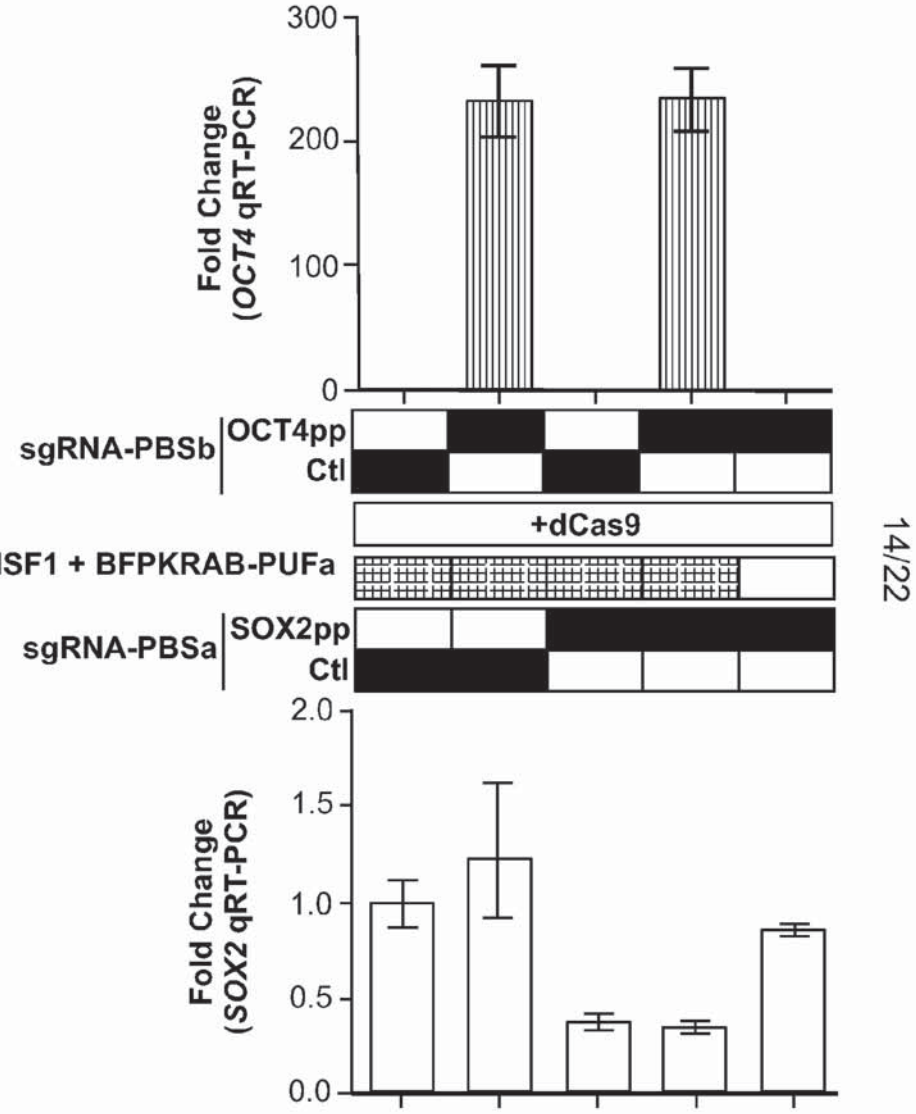

mean GFP fluorescence (4Days)
Fold Change (tdTomato)
25
20
15
10
5
0
PUFc-p65HSF1
sgRNA-PBSc
Tet
Ctl
+dCas9
KRAB-PUFa
sgRNA-PBSa
SV40P1
Ctl
Fold Change (GFP)
1.0
0.5
0.0
p65HSF1
PUFc
x5
PBSc
tdTomato
dCas9
7xTet
KRAB
PUFa
x5
PBSa
EGFP
dCas9
SV40

FIG. 4D


p65HSF1
PUFb
x5
dCas9
PBSb
OCT4


KRAB
PUFa
x5
dCas9
PBSa
SOX2


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

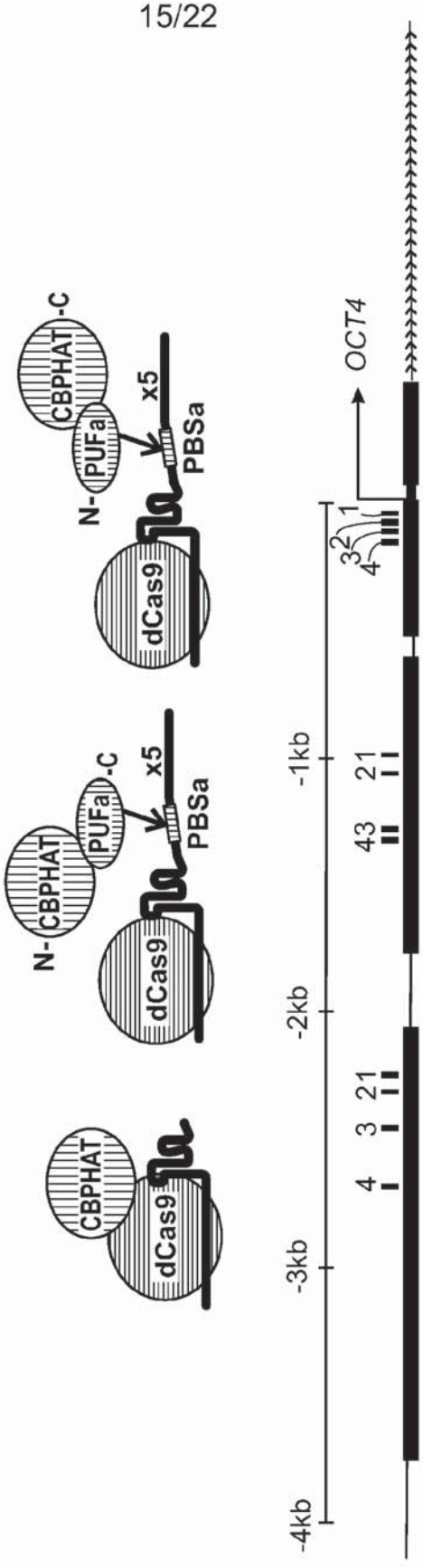

CBPHAT
dCas9
N-
CBPHAT
PUFa
-C
x5
PBSa
dCas9
N-
CBPHAT
PUFa
-C
x5
PBSa
dCas9
-4kb
-3kb
-2kb
-1kb
4
3
21
43
21
3
2
1
4
DE
PE
PP
OCT4

FIG. 5A

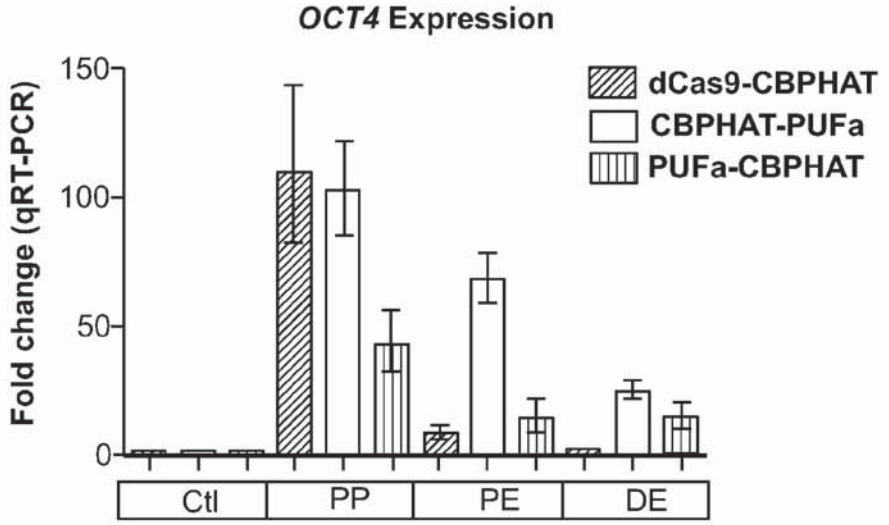

OCT4 Expression
Fold change (qRT-PCR)
150
100
50
0
dCas9-CBPHAT
CBPHAT-PUFa
PUFa-CBPHAT
Ctl
PP
PE
DE

FIG. 5B

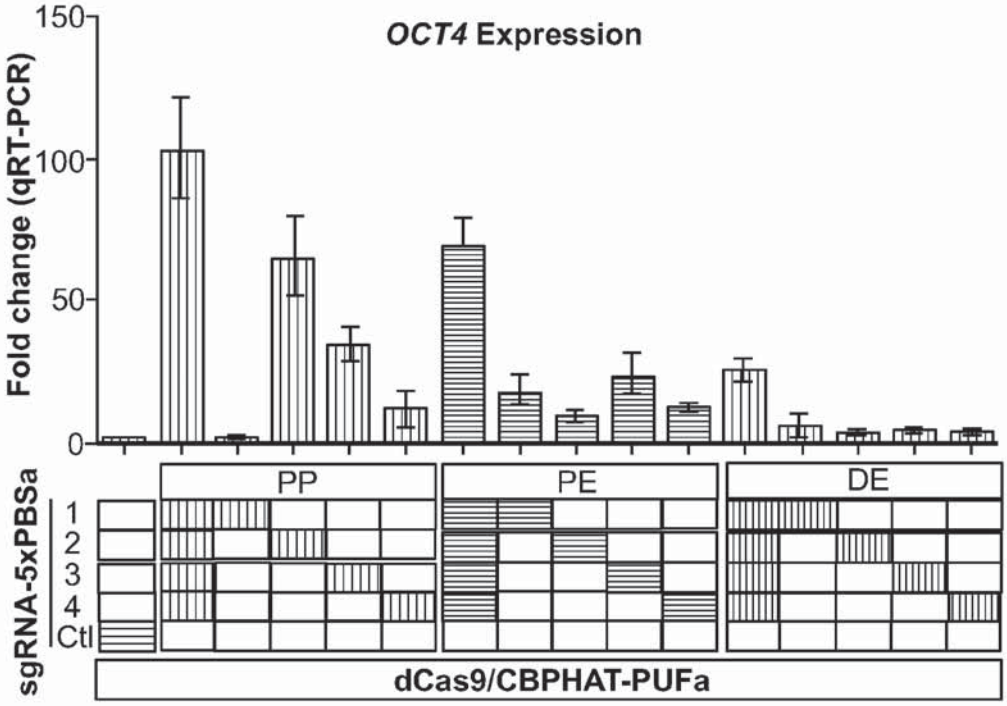

OCT4 Expression
Fold change (qRT-PCR)
150
100
50
0
PP
PE
DE
sgRNA-5xPBSa
1
2
3
4
Ctl
dCas9/CBPHAT-PUFa

FIG. 5C

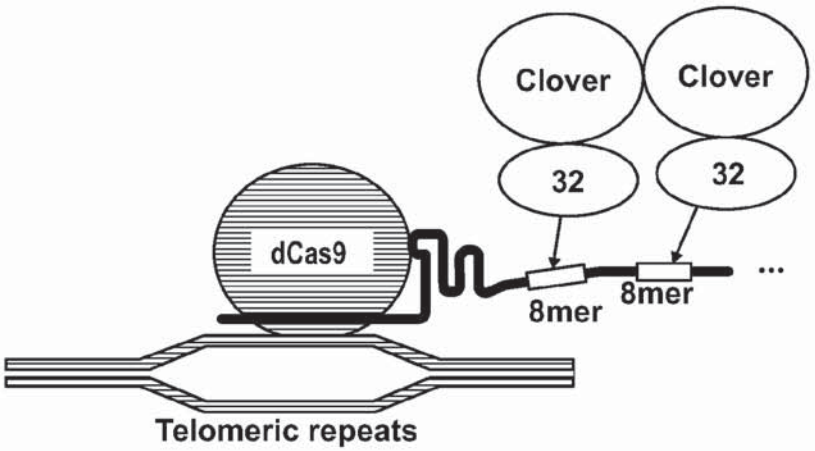


FIG. 6A

FIG. 6B

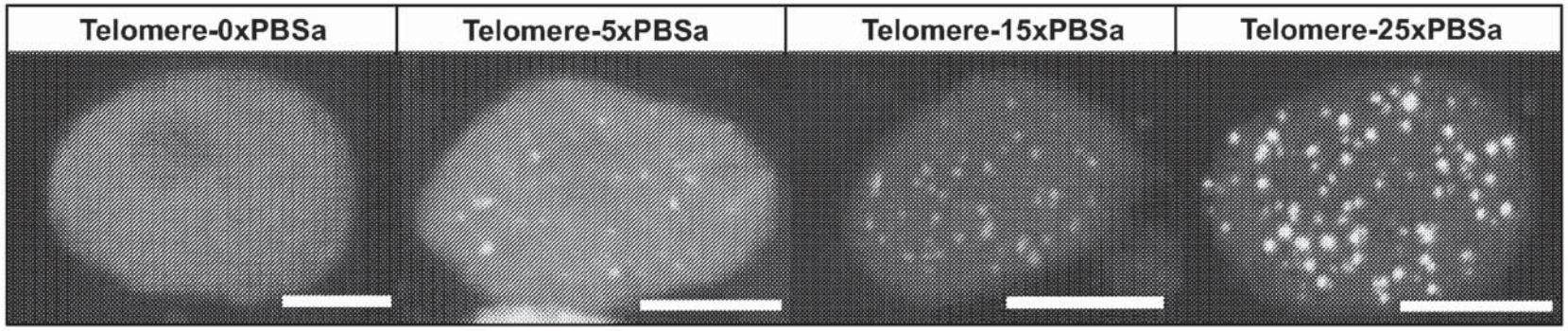

Telomere-0xPBSa
Telomere-5xPBSa
Telomere-15xPBSa
Telomere-25xPBSa

FIG. 6C

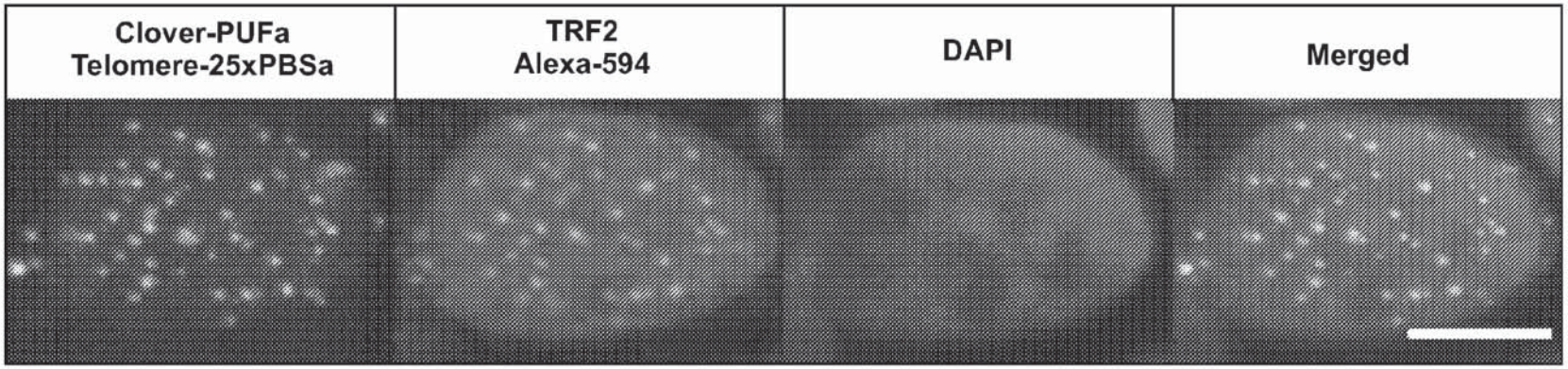

Clover-PUFa
Telomere-25xPBSa
TRF2
Alexa-594
DAPI
Merged

FIG. 6D

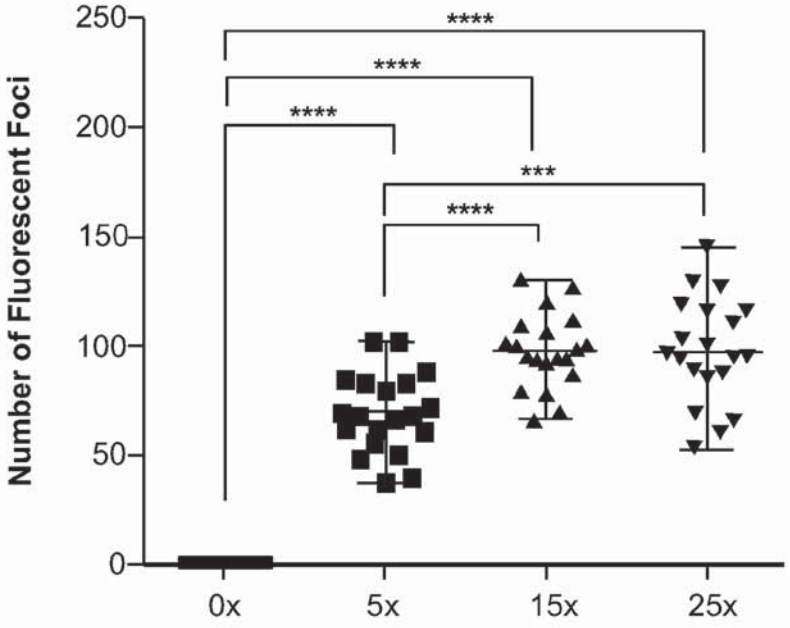

Number of Fluorescent Foci
250
200
150
100
50
0
****
****
****
***
****
0x
5x
15x
25x

FIG. 6E

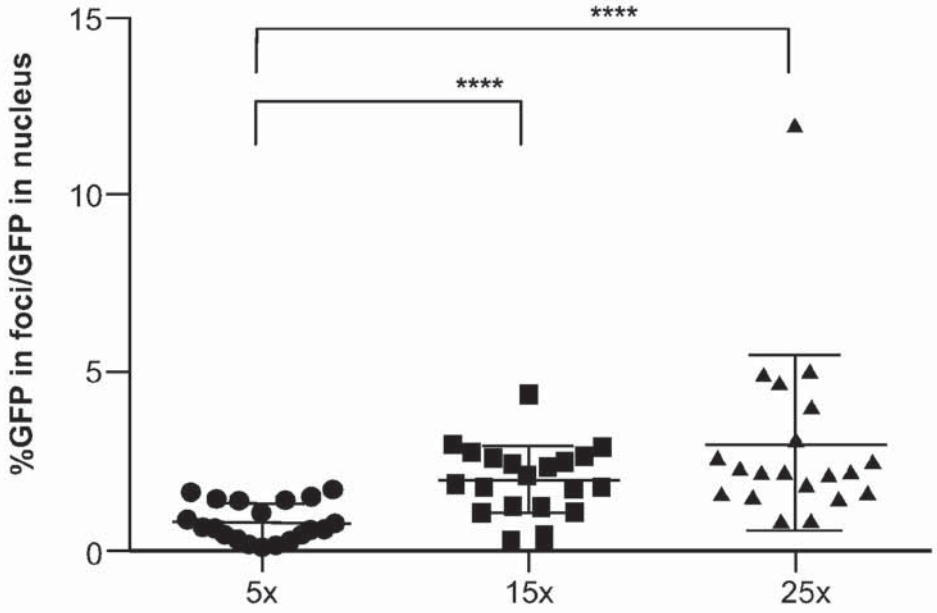

%GFP in foci/GFP in nucleus
15
10
5
0
****
****
5x
15x
25x

FIG. 6F

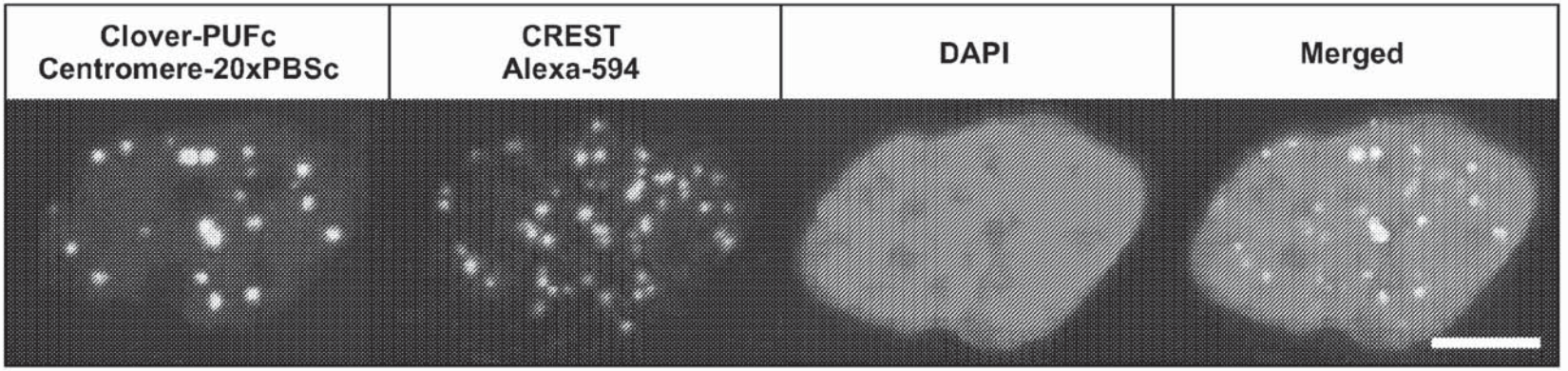
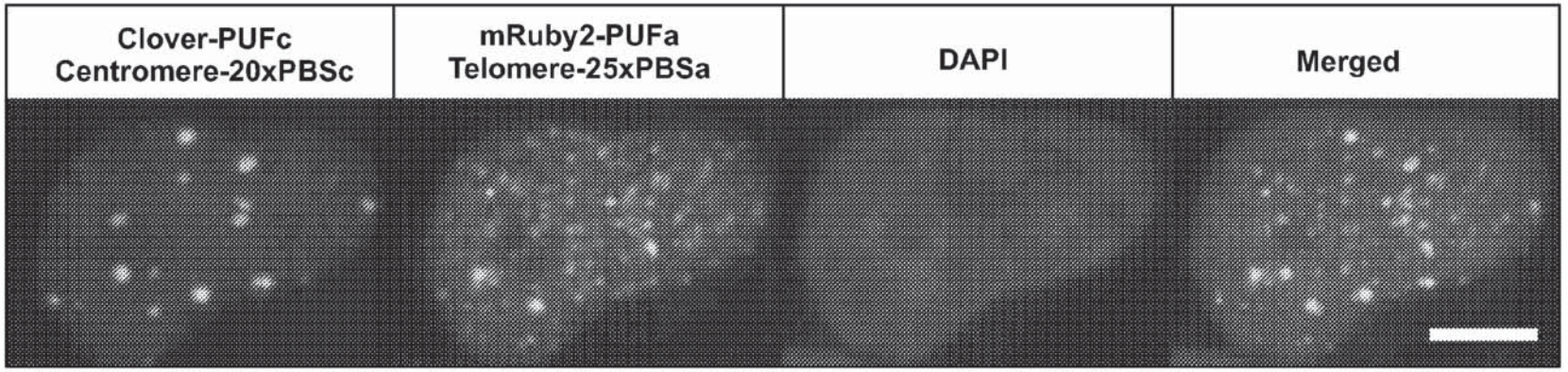

Clover-PUFc
Centromere-20xPBSc
CREST
Alexa-594
DAPI
Merged

FIG. 6G


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

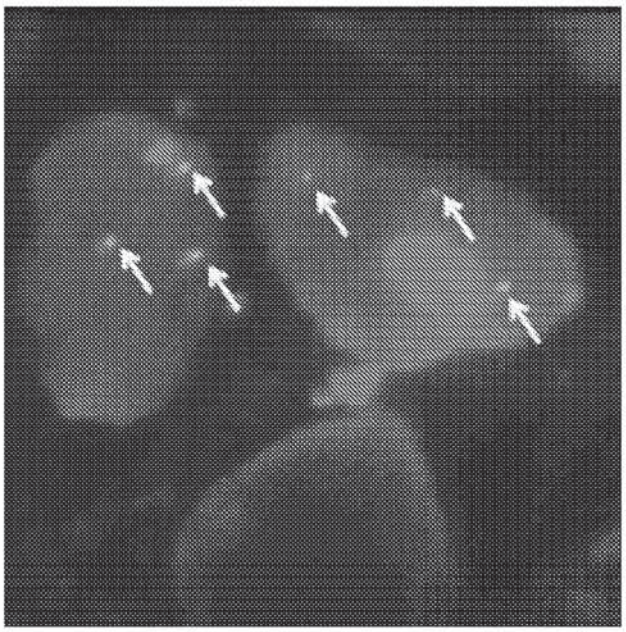


FIG. 7

FIG. 8A

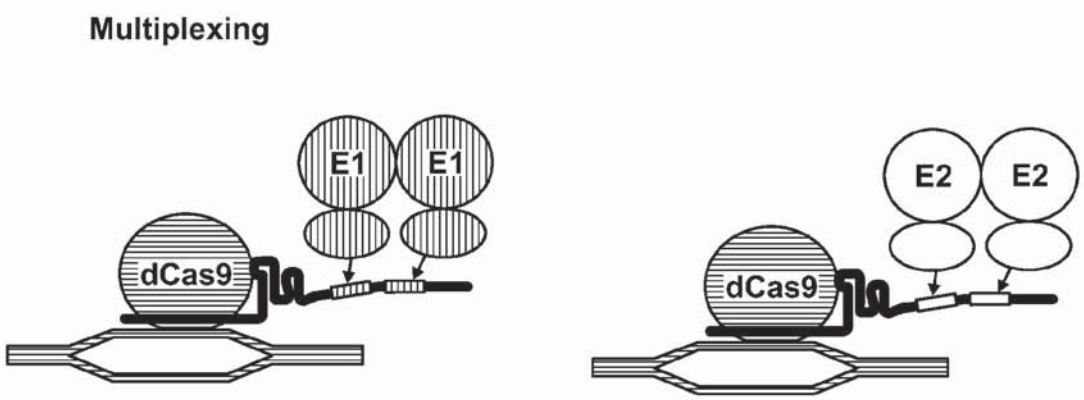


FIG. 8B

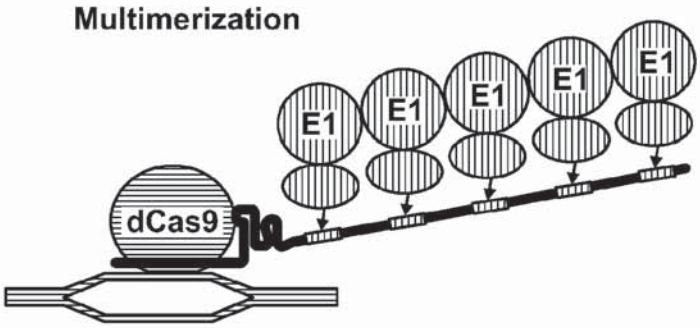


FIG. 8C

