Supplemental Figure Legends

Figure S1. TZAP a novel telomere associated protein. **(A)** and (B) IF-FISH for myc-tagged TZAP in TRF2$^{F/F}$ Rs26^{CRE-ER} MEFs either untreated (-OHT) or 4 days post tamoxifen (+OHT) treatment, U2OS cells, and HeLa 1.2.11 cells. Telomeric PNA probe (green), anti-Myc antibody (green), and DAPI (blue). **(C)** Quantification of % of cells with myc localized at >90% of telomeres in the indicated cells shown in (A) and (B). **(D)** CRISPR/Cas9 gene editing of TZAP in U2OS cells, a schematic of the TZAP locus showing landmarks relevant to CRISPR editing, and DNA sequence of TZAP in the two KO clones. gRNA target region and PAM sequences are indicated in the reference sequence and the deletions are highlighted in each edited allele. (**E)** Quantification of % of cells with TZAP localized at telomeres in parental U2OS clones as well as two CRISPR KO clones. **(F)** Western Blot for endogenous TZAP and for FLAG **(G)** Anti-Flag Immunoprecipitation (IP) was performed from lysates of 293t cells transfected with the indicated flag-tagged and myc-tagged constructs, and from lysates of doxycycline-inducible flag-TZAP expressing U2OS cells transfected with the indicated myc-tagged constructs **(H)**. **(I)** PCR of tamoxifen-induced CRE recombination of floxed alleles in TRF1$^{F/F}$TRF2$^{F/F}$ Rs26^{CRE-ER} MEFs shown in Figure **1E**.

Figure S2. The Zinc Finger Domains (Znc) of TZAP mediate direct interaction with telomeric repeats. **(A)** PCR indicating tamoxifen-induced CRE recombination of floxed TRF2 in TRF2$^{F/F}$ Rs26^{CRE-ER} MEFs shown in Figure 2A-D. **(B)** Quantification of telomeres engaged in end-to-end chromosome fusions analyzed by metaphase-FISH shown in Figure 2C. **(C)** Native gel showing the telomeric g-overhang in cells expressing the indicated constructs. MboI digested genomic DNA was resolved on a pulsed field gel and probed using a radioactive telomeric probe. DNA was isolated form untreated cells (-OHT) or 4 days post OHT treatment (+OHT). **(D)** Immunoblot of the indicated Myc-tagged proteins expressed in TRF2$^{F/F}$ Rs26^{CRE-ER} MEFs shown in Figure 2A-D. **(E)** Schematic of the truncation alleles of flag-tagged TZAP used (**F)** IF for localization of the indicated flag-tagged alleles transiently transfected in U2OS cells. Anti-TRF2 (red), anti-Flag (green), and DAPI (blue) **(G)** IF for localization of indicated flag-tagged constructs in stably-infected HeLa 1.2.11 cells and U2OS cells. Anti-TRF2 (red), anti-flag (green), and DAPI (blue). **(H)** Quantification of the data shown in G, indicating the % of cells with Flag at >90% of telomeres. **(I)** Immunoblots of flag-tagged proteins in U2OS and HeLa cells stably expressing the indicated constructs.

Figure S3. Zinc finger domains 9-11 are required and sufficient for binding telomeres. **(A)** Schematic of the myc-tagged constructs used. **(B)** IF for telomeric localization of the indicated myc-tagged constructs in $TRF2^{F/F}$ $Rs26^{CRE-ER}$ MEFs 4 days post tamoxifen treatment (+OHT). % of cells with myc localized at >90% of telomeres shown in merged image. Anti-TRF1 (red), anti-myc (green), and DAPI (blue) **(C)** MboI digested genomic DNA was resolved on a pulsed field gel, denatured and probed using radioactive telomeric probe. DNA was isolated from $TRF2^{F/F}$ $Rs26^{CRE-ER}$ MEFs expressing the indicated myc-tagged constructs either untreated (-OHT), or at 4 days post tamoxifen treatment (+OHT). **(D)** Immunoblot of myc-tagged proteins. **(E)** Polyacrylamide electrophoresis gel showing purification of recombinant HisMBP and HisMBP-$TZAP^{znf9-11}$. Gel was stained with Coomassie brilliant blue. **(F)** EMSA showing binding of recombinant HisMBP-$TZAP^{znf9-11}$ to [γ-32P] labeled Telo-6 probe in the presence of a 100-fold (ds$[TTAGG]_n$: n= 6, 3, 1, 0; ssTelG, ssTelC) or 10-fold (plasmids: $[TTAGG]_{270}$, pSP73) molar excess of different DNA templates as competitors. Lane 1, ^{32}P-labeled Telo-6 probe alone.

Figure S4. TZAP binding to telomeres is inhibited by elevated TRF2 levels. **(A)** IF for localization of doxycycline-inducible Flag-TZAP at telomeres in U2OS cells with and without overexpression of Myc-TRF2. Anti-TRF1 (red), anti-Flag (green), and Myc-TRF2 (blue). **(B)** Immunoblots of indicated Myc-tagged and Flag-tagged proteins expressed in cells shown in Figure 3D-E. **(C)** Immunoblots of the indicated flag-tagged proteins expressed in cells shown in Figure 3F-G. **(D)** IF-FISH for localization of either flag-TRF1 or flag-TZAP in MEFs with and without overexpression of myc-TRF2. Telomeric PNA probe (red), anti-Flag (green), and anti-Myc (blue). **(E)** Quantification of % of cells with flag at >90% of telomeres shown in D. **(F)** Immunoblots of the indicated flag-tagged and myc-tagged proteins expressed in cells.

Figure S5. TZAP preferentially binds long telomeres. **(A)** IF for localization of either Flag-TRF1 (bottom panels) or Flag-TZAP (top panels) ectopically expressed in various mammalian cell lines. Anti-TRF2 (red), anti-Flag (green), and DAPI (blue). Cells are arranged in order of increasing average telomere length from left to right (see E for details). **(B)** Table indicating the cell lines analyzed: the transformation status, telomerase expression, ALT status, average telomere length, percentage of cells with either Flag-TRF1 or Flag-TZAP at >90% of telomeres.

Figure S6. TZAP contributes to telomere length homeostasis promoting telomere trimming. **(A)** T-circle analysis of U2OS cells overexpressing TZAP or empty vector at 6 days post infection. **(B)** T-circle analysis of U2OS cells transduced with the indicated siRNA at 3 days post treatment.

Samples not supplemented with the Phi polymerase represent a negative control to assess the specificity of the reaction. **(C)** Quantification of T circle analysis shown in A. **(D)** Quantificaiton of T circle analyses in cells treated as indicated **(E)** C-circle analysis of GM847 cells overexpressing TZAP or empty vector at 6 days post infection. (**F**) C-circle analysis of U2OS cells and GM847 cells transducted with the indicated siRNAs at 3 days post treatment. (**G**) Quantification of C circles of GM847 cells either overexpressing the indicated constructs or transduced with the indicated siRNAs. **(H)** Quantificaiton of C circles of U2OS cells either overexressing the indicated constructs or transduced with the indicated siRNAs **(I)** IF of PML (red), Myc-TZAP (green), and TRF2 (blue) of U2OS cells and upon infection with Myc-TZAP at the indicated time points. **(J)** IF of PML(red), Myc-TZAP (green), and TRF2 (blue) of GM847 cells 6 days post infection with Myc-TZAP (**K**) Quantification of APBs as defined by colocalization of PML at TRF2 of the data shown in I and J.

Figure S7. Telomere are elongated in CRISPR-mediated TZAP-/- mES clones. **(A)** Schematic of the sgRNA target sequence at TZAP exon2 and the HDR repair template containing a STOP cassette flanked by homology regions. **(B)** Schematic of integration of the HDR repair template at the target site and DNA sequence of the three TZAP-/- clones. **(C)** qPCR of mRNA transcript levels in WT and TZAP-/- cells. **(D)** C-circle amplification assay of WT and TZAP-/- cells (clone #1). **(E)** Quantificaiton of C circle amplification shown in D. **(F)** Denaturing gel showing the average telomere length (value indicated within the gel) of parental WT and TZAP-/- #1 cells. **(G)** IF of localization of Myc-TZAP at telomeres in TZAP-/- clones stably expressing Myc-TZAP. Anti-TRF1 (red), anti-Myc (green), and DAPI (blue). **(H)** Q-FISH analysis of mES cells either mock infected or infected with a Myc-TZAP.

Figure S8. Schematic representation of proposed role for TZAP in telomere length homeostasis.

Figure S1

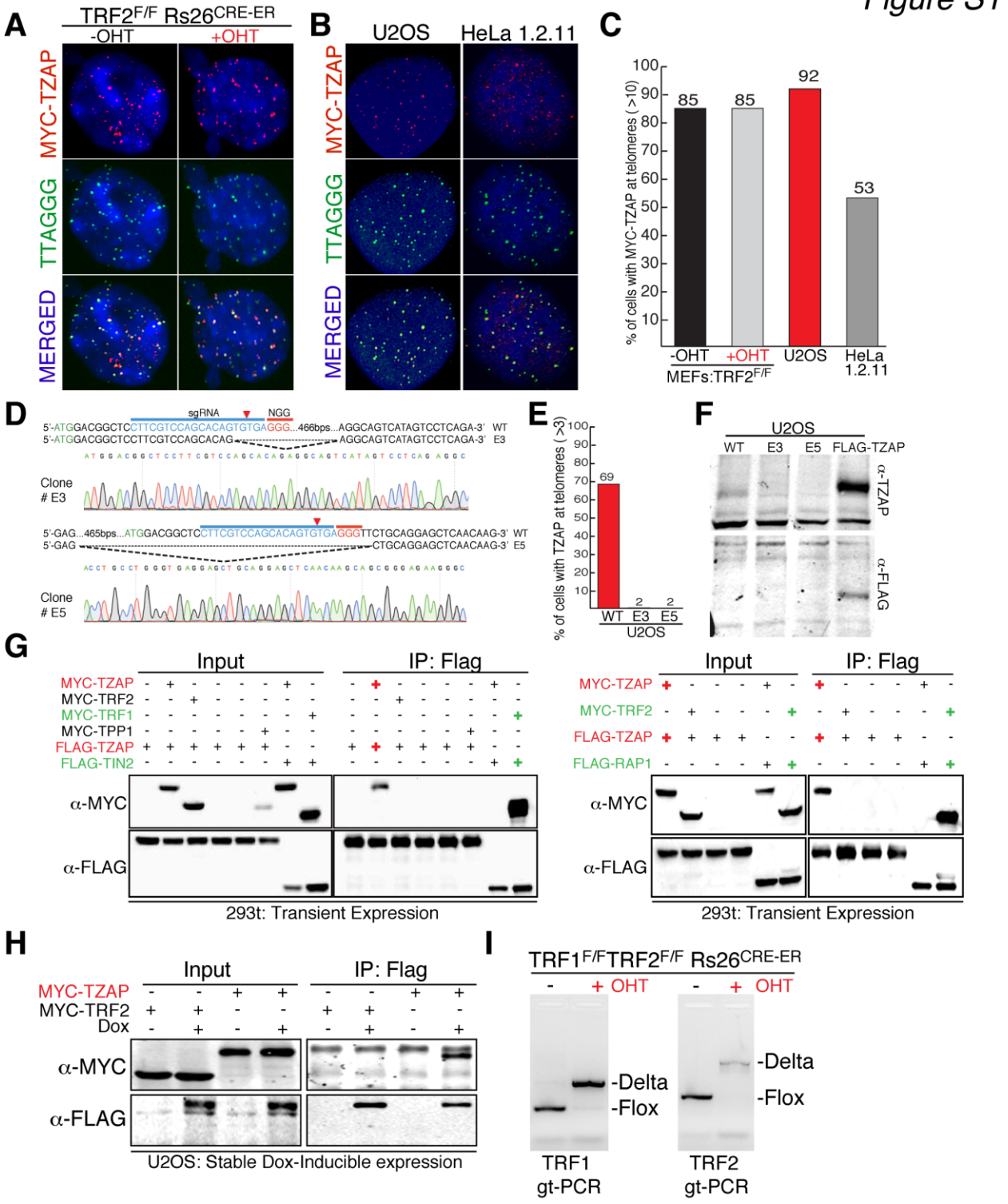

A
TRF2F/F Rs26CRE-ER
-OHT
+OHT
MYC-TZAP
TTAGGG
MERGED
B
U2OS
HeLa 1.2.11
C
% of cells with MYC-TZAP at telomeres (>10)
85
85
92
53
-OHT
+OHT
U2OS
HeLa 1.2.11
MEFs:TRF2F/F
D
sgRNA
NGG
5'-ATGGACGGCTCCTTCGTCCAGCACAGTGTGAGGG...466bps...AGGCAGTCATAGTCCTCAGA-3' WT
5'-ATGGACGGCTCCTTCGTCCAGCACAG AGGCAGTCATAGTCCTCAGA-3' E3
Clone # E3
5'-GAG...465bps...ATGGACGGCTCCTTCGTCCAGCACAGTGTGAGGGTTCTGCAGGAGCTCAACAAG-3' WT
5'-GAG CTGCAGGAGCTCAACAAG-3' E5
Clone # E5
E
% of cells with TZAP at telomeres (>3)
69
2
2
WT
E3
E5
U2OS
F
U2OS
WT
E3
E5
FLAG-TZAP
α-TZAP
α-FLAG
G
Input
IP: Flag
MYC-TZAP
MYC-TRF2
MYC-TRF1
MYC-TPP1
FLAG-TZAP
FLAG-TIN2
α-MYC
α-FLAG
293t: Transient Expression
Input
IP: Flag
MYC-TZAP
MYC-TRF2
FLAG-TZAP
FLAG-RAP1
α-MYC
α-FLAG
293t: Transient Expression
H
Input
IP: Flag
MYC-TZAP
MYC-TRF2
Dox
α-MYC
α-FLAG
U2OS: Stable Dox-Inducible expression
I
TRF1F/FTRF2F/F Rs26CRE-ER
- + OHT
- + OHT
-Delta
-Flox
-Delta
-Flox
TRF1
gt-PCR
TRF2
gt-PCR

Figure S2

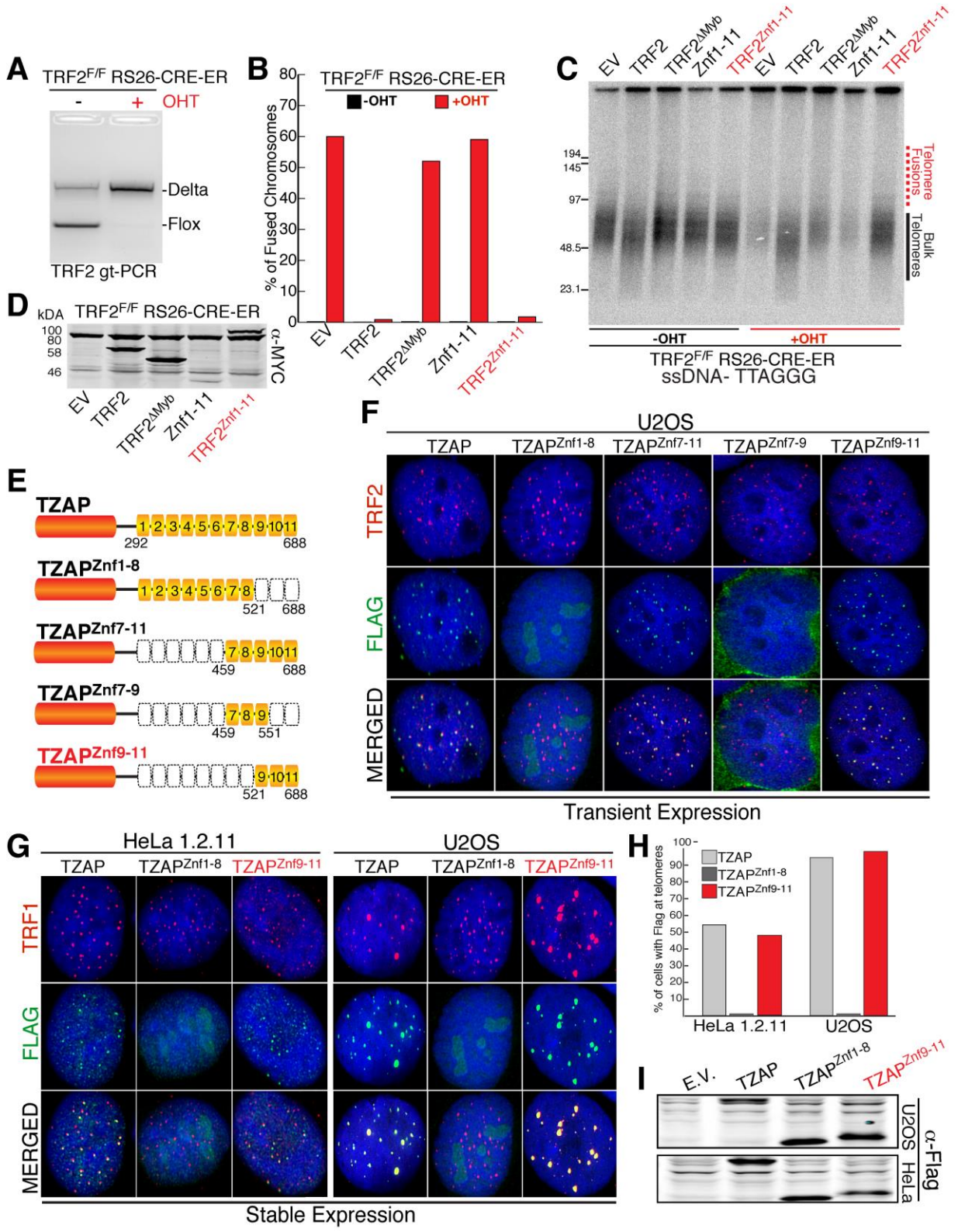

A
TRF2F/F RS26-CRE-ER
- + OHT
-Delta
-Flox
TRF2 gt-PCR
B
TRF2F/F RS26-CRE-ER
-OHT +OHT
% of Fused Chromosomes
EV TRF2 TRF2ΔMyb Znf1-11 TRF2Znf1-11
C
EV TRF2 TRF2ΔMyb Znf1-11 TRF2Znf1-11 EV TRF2 TRF2ΔMyb Znf1-11 TRF2Znf1-11
194
145
97
48.5
23.1
Telomere Fusions
Bulk Telomeres
-OHT +OHT
TRF2F/F RS26-CRE-ER
ssDNA- TTAGGG
D
kDA TRF2F/F RS26-CRE-ER
100
80
58
46
α-MYC
EV TRF2 TRF2ΔMyb Znf1-11 TRF2Znf1-11
E
TZAP
292 688
TZAPZnf1-8
521 688
TZAPZnf7-11
459 688
TZAPZnf7-9
459 551
TZAPZnf9-11
521 688
F
U2OS
TZAP TZAPZnf1-8 TZAPZnf7-11 TZAPZnf7-9 TZAPZnf9-11
TRF2
FLAG
MERGED
Transient Expression
G
HeLa 1.2.11
U2OS
TZAP TZAPZnf1-8 TZAPZnf9-11 TZAP TZAPZnf1-8 TZAPZnf9-11
TRF1
FLAG
MERGED
Stable Expression
H
TZAP
TZAPZnf1-8
TZAPZnf9-11
% of cells with Flag at telomeres
HeLa 1.2.11
U2OS
I
E.V. TZAP TZAPZnf1-8 TZAPZnf9-11
U2OS
HeLa
α-Flag

Figure S3

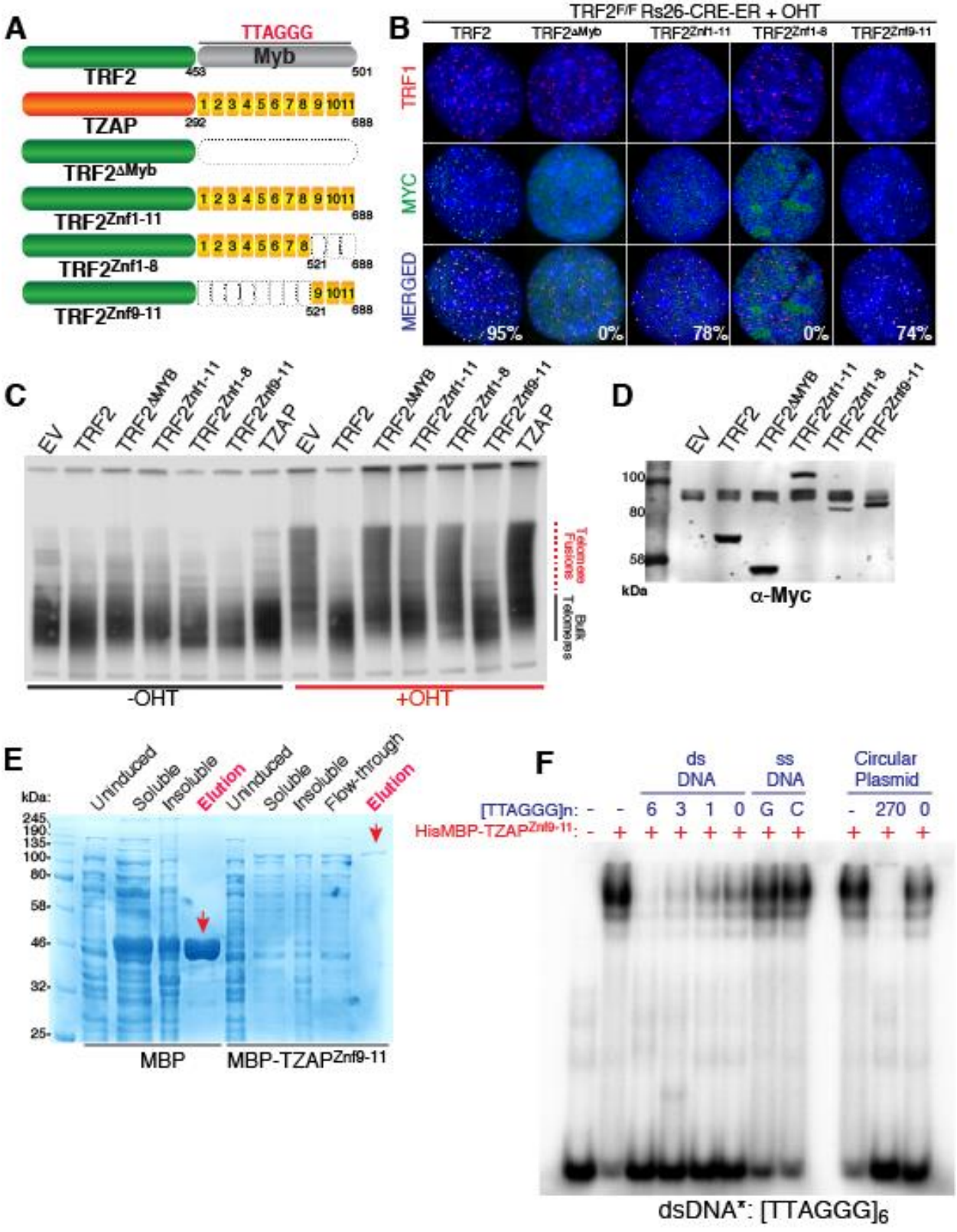

A
TTAGGG
Myb
TRF2
453
501
1 2 3 4 5 6 7 8 9 1011
TZAP
292
688
TRF2ΔMyb
TRF2Znf1-11
TRF2Znf1-8
521
TRF2Znf9-11
B
TRF2F/F Rs26-CRE-ER + OHT
TRF2
TRF2ΔMyb
TRF2Znf1-11
TRF2Znf1-8
TRF2Znf9-11
TRF1
MYC
MERGED
95%
0%
78%
0%
74%
C
EV
TRF2
TRF2ΔMYB
TRF2Znf1-11
TRF2Znf1-8
TRF2Znf9-11
TZAP
Telomere Fusions
Bulk Telomeres
-OHT
+OHT
D
100
80
58
kDa
α-Myc
E
Uninduced
Soluble
Insoluble
Elution
Flow-through
kDa:
245
190
135
100
80
58
46
32
25
MBP
MBP-TZAPZnf9-11
F
ds DNA
ss DNA
Circular Plasmid
[TTAGGG]n: - - 6 3 1 0 G C - 270 0
HisMBP-TZAPZnf9-11: - + + + + + + + + + +
dsDNAx: [TTAGGG]6

Figure S4

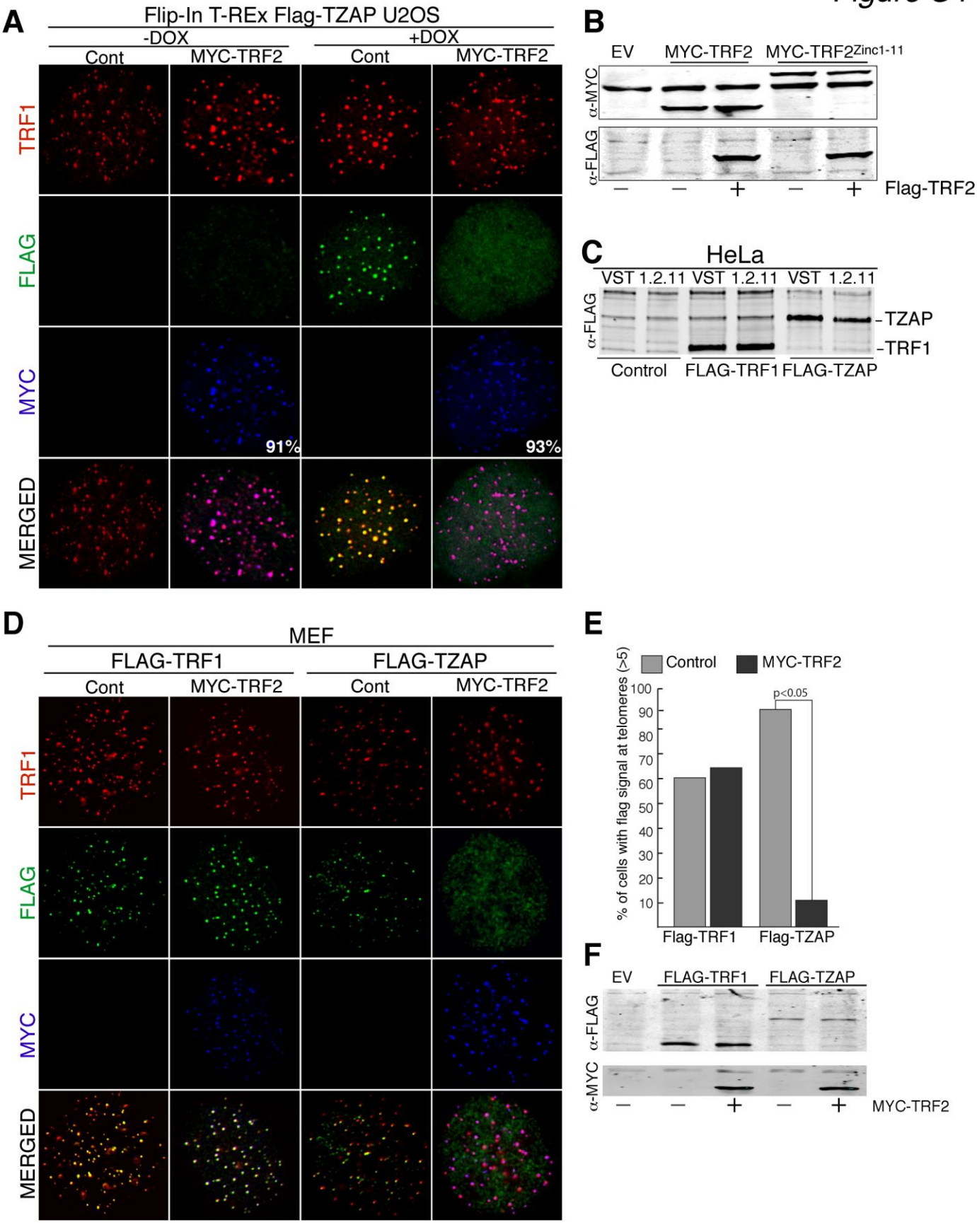
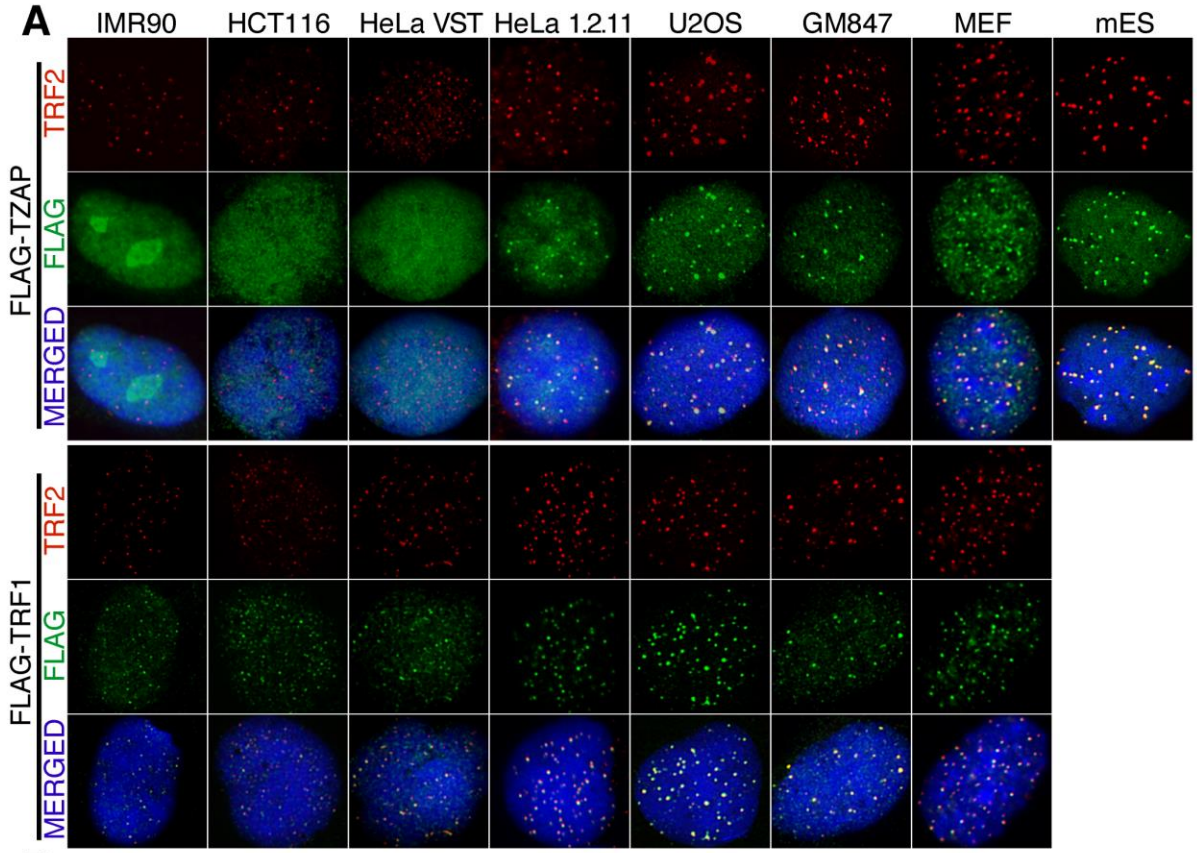

A
Flip-In T-REx Flag-TZAP U2OS
-DOX
+DOX
Cont
MYC-TRF2
Cont
MYC-TRF2
TRF1
FLAG
MYC
MERGED
91%
93%
B
EV
MYC-TRF2
MYC-TRF2Zinc1-11
α-MYC
α-FLAG
– – + – +
Flag-TRF2
C
HeLa
VST 1.2.11 VST 1.2.11 VST 1.2.11
α-FLAG
-TZAP
-TRF1
Control
FLAG-TRF1
FLAG-TZAP
D
MEF
FLAG-TRF1
FLAG-TZAP
Cont
MYC-TRF2
Cont
MYC-TRF2
TRF1
FLAG
MYC
MERGED
E
Control
MYC-TRF2
% of cells with flag signal at telomeres (>5)
100
90
80
70
60
50
40
30
20
10
p<0.05
Flag-TRF1
Flag-TZAP
F
EV
FLAG-TRF1
FLAG-TZAP
α-FLAG
α-MYC
– – + – +
MYC-TRF2

Figure S5



B

	IMR90	HCT116	HeLa VST	HeLa 1.2.11	U2OS	GM847	MEF	mES
	Primary	Transformed	Transformed	Transformed	Transformed	Transformed	Primary	Primary
Telomerase	-	+	+	+	-	-	+	+
ALT	-	-	-	-	+	+	-	-
Tel Length (kb)	~5	~5	~5	~20	~4-45	~4-45	~40	~100
% TZAP +ve	0	0	0	53	92	75	90	85
% TRF1 +ve	100	73	75	78	98	89	61	

Figure S6

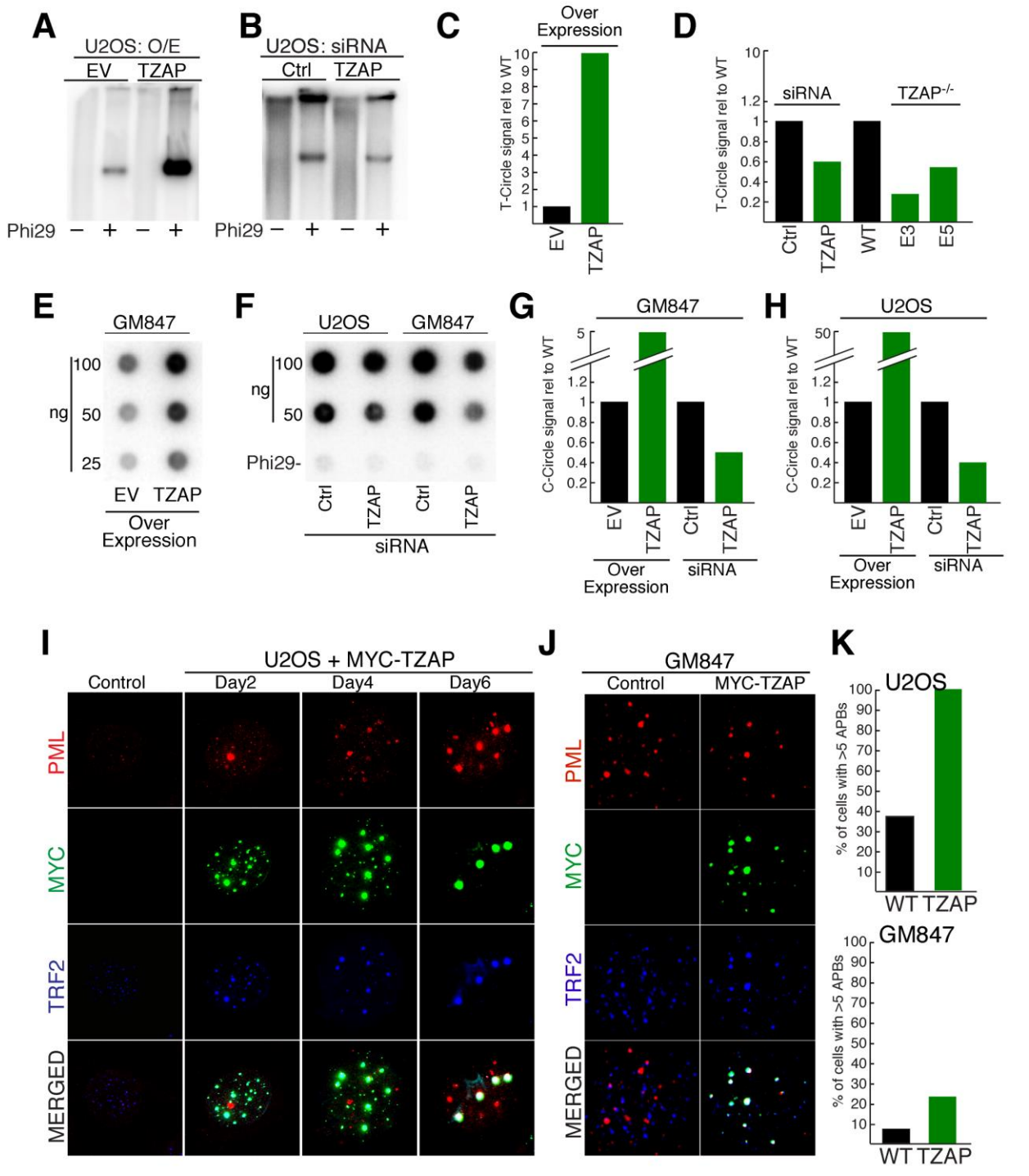
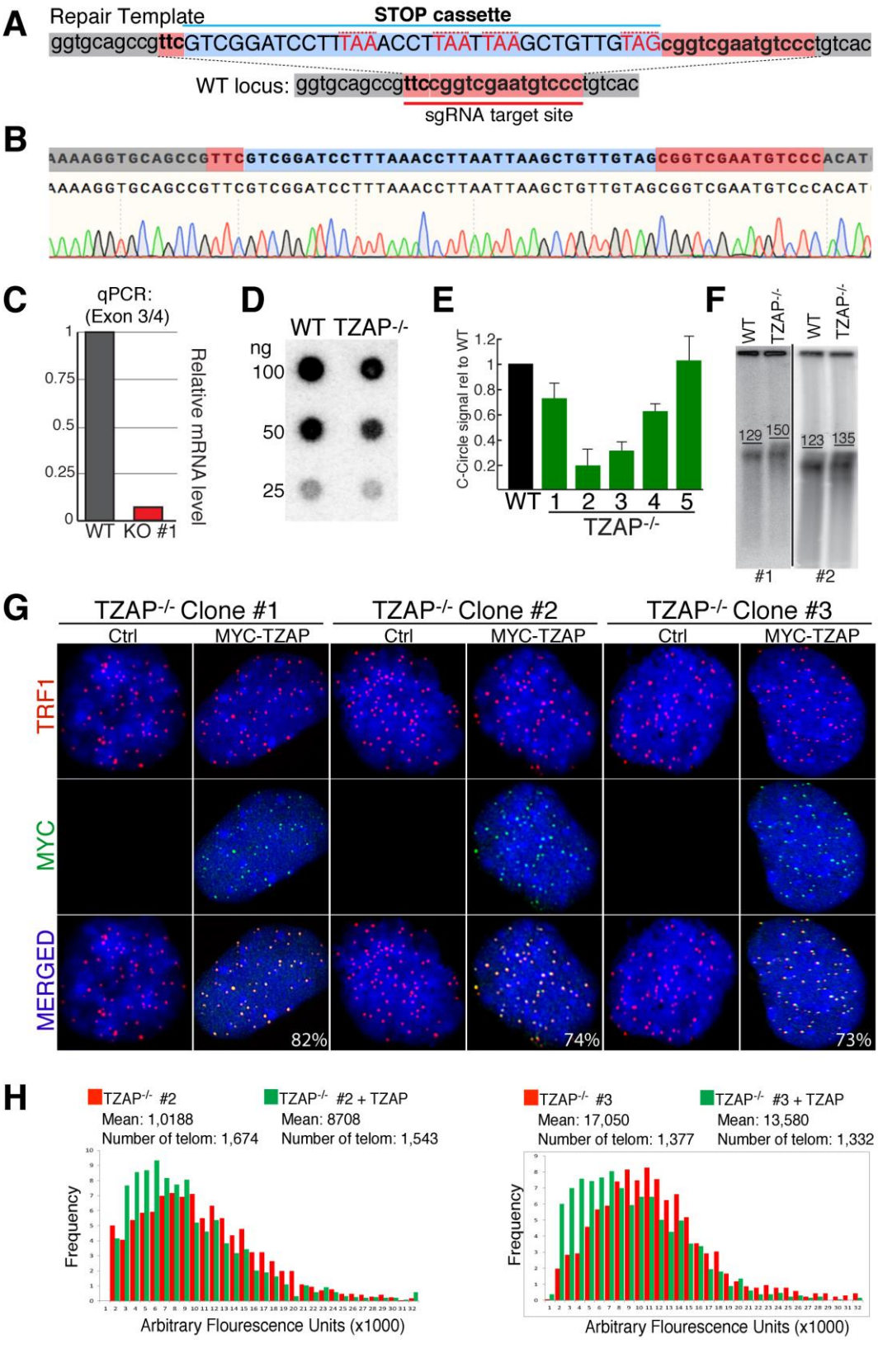

A
U2OS: O/E
EV
TZAP
Phi29 – + – +
B
U2OS: siRNA
Ctrl
TZAP
Phi29 – + – +
C
Over Expression
T-Circle signal rel to WT
EV
TZAP
D
T-Circle signal rel to WT
siRNA
TZAP-/-
Ctrl
TZAP
WT
E3
E5
E
GM847
ng
100
50
25
EV TZAP
Over Expression
F
U2OS
GM847
ng
100
50
Phi29-
Ctrl
TZAP
Ctrl
TZAP
siRNA
G
GM847
C-Circle signal rel to WT
EV
TZAP
Ctrl
TZAP
Over Expression
siRNA
H
U2OS
C-Circle signal rel to WT
EV
TZAP
Ctrl
TZAP
Over Expression
siRNA
I
U2OS + MYC-TZAP
Control
Day2
Day4
Day6
PML
MYC
TRF2
MERGED
J
GM847
Control
MYC-TZAP
PML
MYC
TRF2
MERGED
K
U2OS
% of cells with >5 APBs
WT TZAP
GM847
% of cells with >5 APBs
WT TZAP

Figure S7


A
Repair Template
STOP cassette
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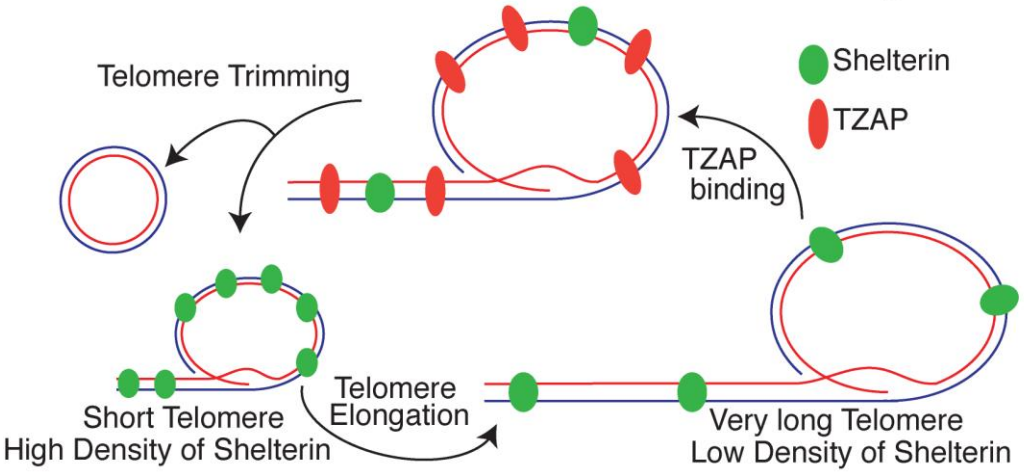
WT locus: ggtgcagccgttccggtcgaatgtcccctgtcac
sgRNA target site
B
C
qPCR:
(Exon 3/4)
Relative mRNA level
WT KO #1
D
WT TZAP-/-
ng
100
50
25
E
C-Circle signal rel to WT
WT 1 2 3 4 5
TZAP-/-
F
WT
TZAP-/-
129 150
123 135
#1
#2
G
TZAP-/- Clone #1
TZAP-/- Clone #2
TZAP-/- Clone #3
Ctrl
MYC-TZAP
TRF1
MYC
MERGED
82%
74%
73%
H
TZAP-/- #2
Mean: 1,0188
Number of telom: 1,674
TZAP-/- #2 + TZAP
Mean: 8708
Number of telom: 1,543
TZAP-/- #3
Mean: 17,050
Number of telom: 1,377
TZAP-/- #3 + TZAP
Mean: 13,580
Number of telom: 1,332
Frequency
Arbitrary Flourescence Units (x1000)

Figure S8


Telomere Trimming
Shelterin
TZAP
TZAP
binding
Short Telomere
High Density of Shelterin
Telomere
Elongation
Very long Telomere
Low Density of Shelterin