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Supplemental Material to:

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TopoIIα prevents telomere fragility and formation of ultra thin DNA bridges during mitosis through TRF1-dependent binding to telomeres

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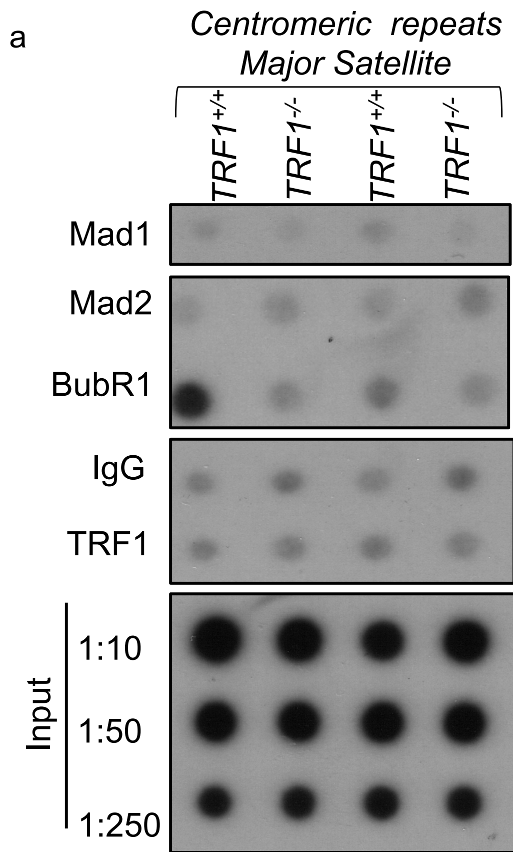
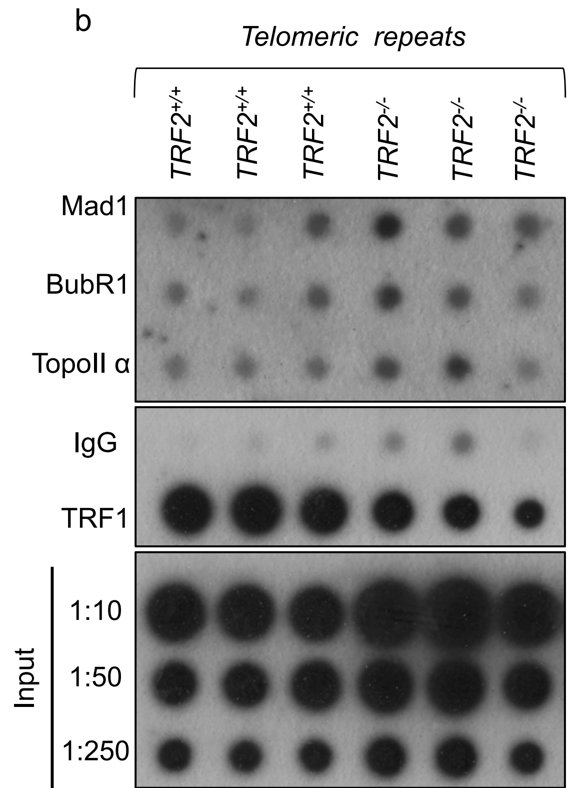
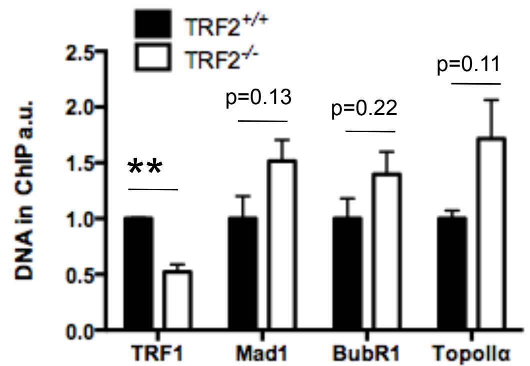
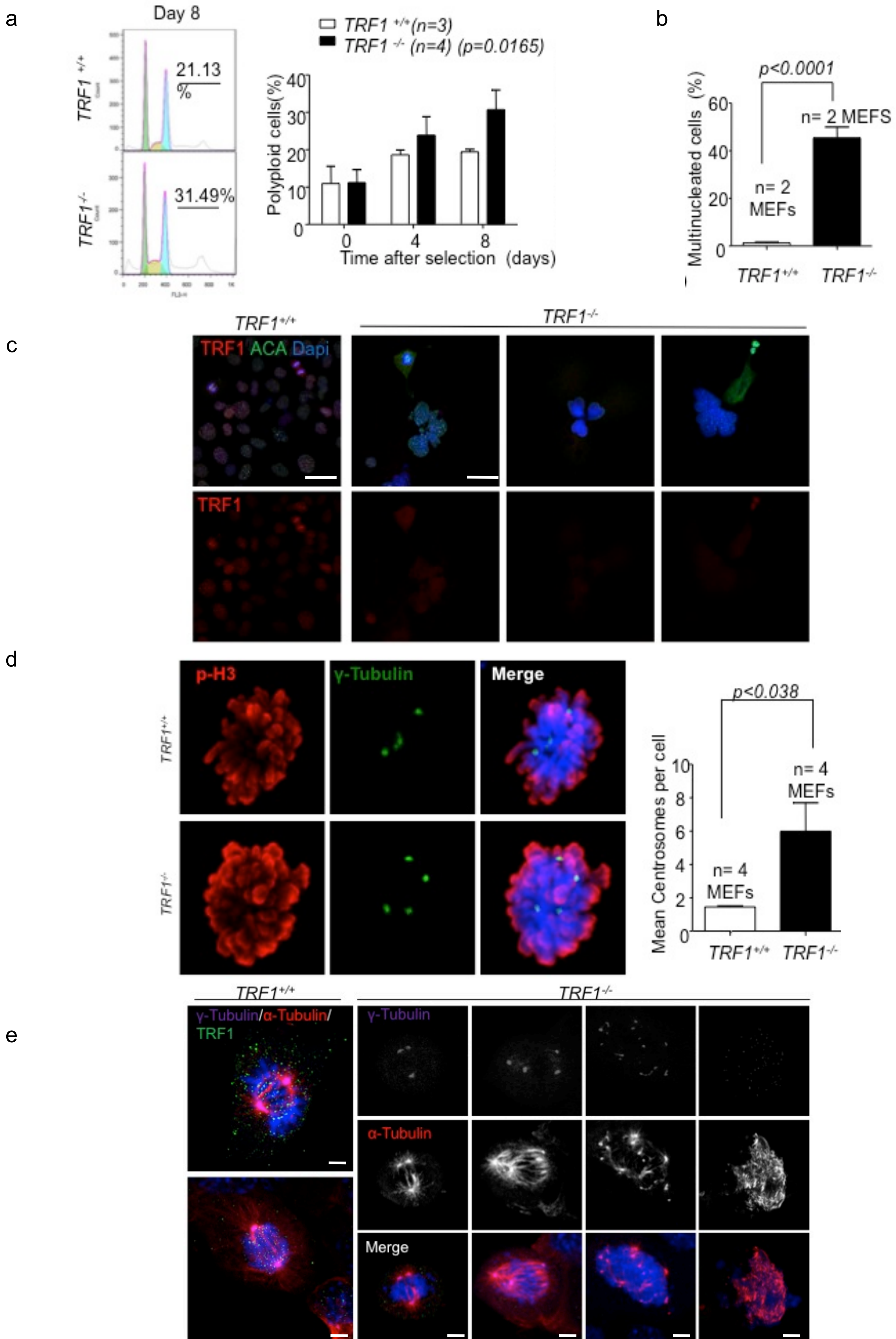
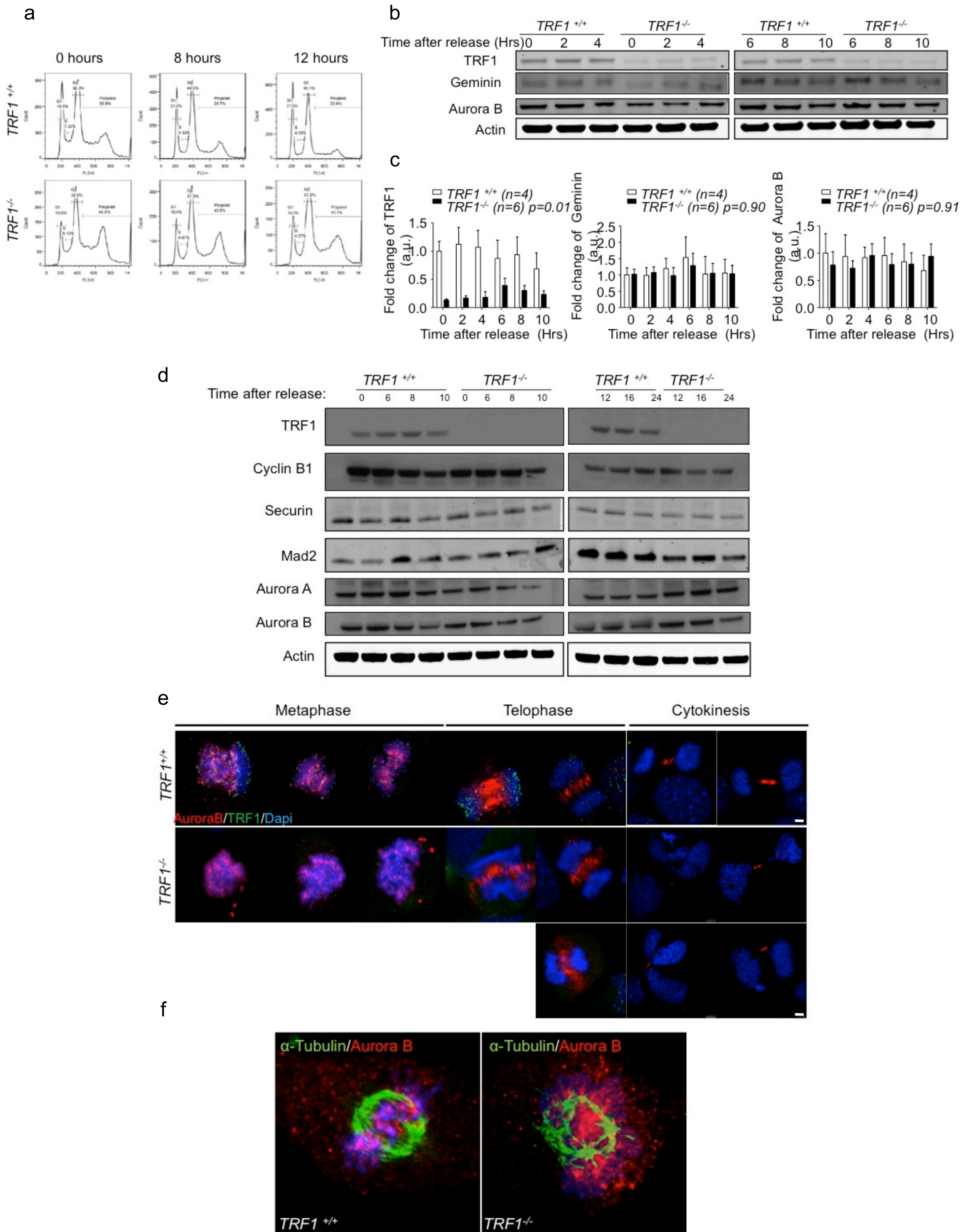

a
Centromeric repeats
Major Satellite
TRF1+/+
TRF1-/-
TRF1+/+
TRF1-/-
Mad1
Mad2
BubR1
IgG
TRF1
Input
1:10
1:50
1:250

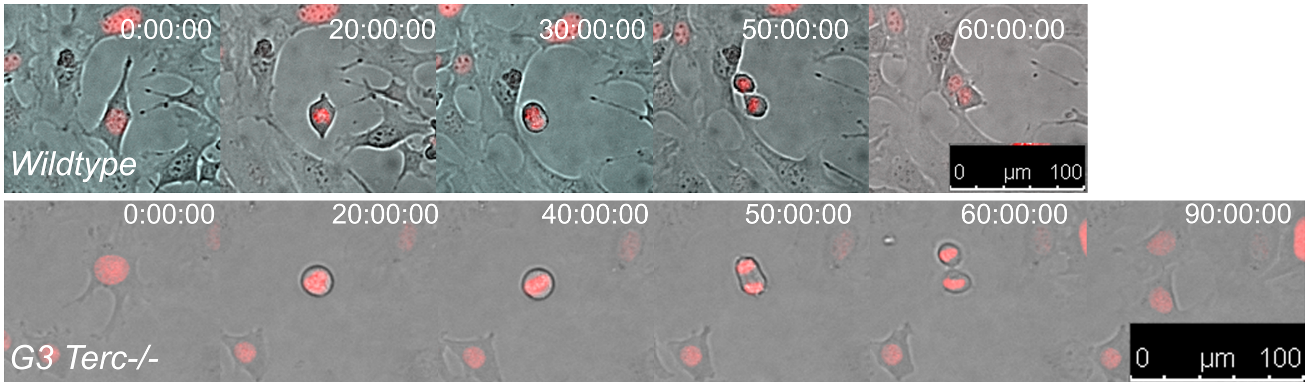

b
Telomeric repeats
TRF2+/+
TRF2+/+
TRF2+/+
TRF2-/-
TRF2-/-
TRF2-/-
Mad1
BubR1
TopoII α
IgG
TRF1
Input
1:10
1:50
1:250


TRF2+/+
TRF2-/-
DNA in ChIP a.u.
2.5
2.0
1.5
1.0
0.5
0.0
**
p=0.13
p=0.22
p=0.11
TRF1
Mad1
BubR1
TopoIIα

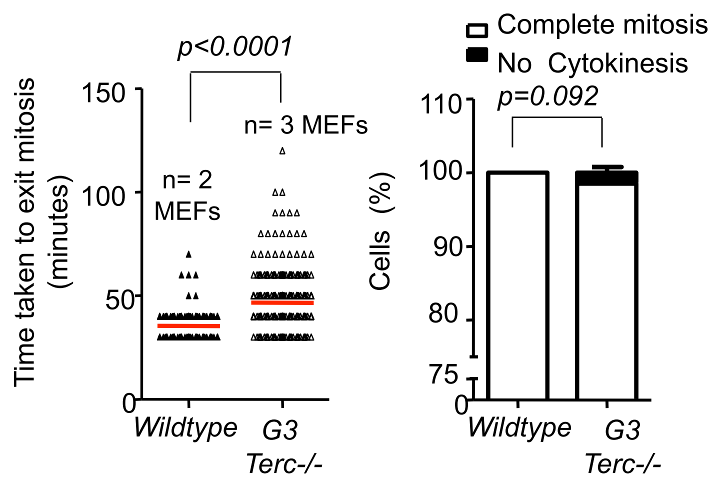

a
Day 8
TRF1 +/+
21.13 %
TRF1 -/-
31.49%
TRF1 +/+ (n=3)
TRF1 -/- (n=4) (p=0.0165)
Polyploid cells(%)
Time after selection (days)
b
p<0.0001
Multinucleated cells (%)
n= 2 MEFS
n= 2 MEFs
TRF1+/+
TRF1-/-
c
TRF1 ACA Dapi
TRF1
d
p-H3
γ-Tubulin
Merge
p<0.038
Mean Centrosomes per cell
n= 4 MEFs
e
γ-Tubulin/α-Tubulin/TRF1
γ-Tubulin
α-Tubulin
Merge


a
0 hours
8 hours
12 hours
TRF1 +/+
TRF1-/-
b
TRF1 +/+
TRF1-/-
Time after release (Hrs)
TRF1
Geminin
Aurora B
Actin
c
TRF1 +/+ (n=4)
TRF1-/- (n=6) p=0.01
TRF1-/- (n=6) p=0.90
TRF1-/- (n=6) p=0.91
Fold change of TRF1 (a.u.)
Fold change of Geminin (a.u.)
Fold change of Aurora B (a.u.)
Time after release (Hrs)
d
Time after release:
TRF1
Cyclin B1
Securin
Mad2
Aurora A
Aurora B
Actin
e
Metaphase
Telophase
Cytokinesis
AuroraB/TRF1/Dapi
f
α-Tubulin/Aurora B

a



b



Supplementary Figure 1 (a) ChIP of *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$*p53*$^{-/-}$ MEFs using specific antibodies against Mad1, Mad2, BubR1 and TRF1 probed with centromeric major satellite probe. **(b)** ChIP of *Trf2*$^{+/+}$ and *Trf2*$^{-/-}$*p53*$^{-/-}$ MEFs using specific antibodies against Mad1, BubR1, TopoIIα and TRF1. The amount of immunoprecipitaded telomere repeats was normalised to the amount of telomere repeats present in the chromatin fraction unbound to preimmune serum. n, independent MEFs used. Bars represent the average between replicates; standard error is used. Quantification of ChIP values is shown in the panel below. A Students's t test was used to calculate statistical significance. Representative Mad1, TopoIIα, BubR1 and TRF1 with MEFs of the indicated genotypes are shown in the bottom panel.

Supplementary Figure 2 TRF1 knockout cells display a mitotic phenotype**. (a)** Representative example of FACS histogram 8 days post selection. Right panel quantification of the percentage of polyploidy cells at the indicated time points post selection. Statistical significance was determined using a two way ANOVA giving a p value of 0.0165. **(b)** Quantification of the percentage of multinucleated cells in two independent *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs. Error bars represent standard error. Fisher exact test was used for statistical analysis and p values are indicated. **(c)** Representative images of immunofluorescence staining in *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs using TRF1 antibody (red), anti centromere antibody (green). Nuclei are counterstained with Dapi (blue). Bars = 50μM **(d)** Representative images of *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs stained with γ-Tubulin (red) and TRF1 (purple). Nuclei are counterstained with Dapi (blue). Right panel: quantification of the mean number of centrosomes per cell of four independent *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs. **(e)** Representative images of *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs stained with γ-Tubulin (red), α-Tubulin (red) and TRF1 (purple) to show that multiple centrosomes are functional and not due to centrosomal fragmentation. Bars = 10μM.

Supplementary Figure 3 TRF1 depletion does not affect APC target stability. **(a)** Representative example of FACS histograms over time upon release from thymidine double block of *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$*p53*$^{-/-}$ MEFs. **(b-d)** No abnormal stabilization of mitotic APC targets in Trf1$^{-/-}$ MEFs. Trf1$^{+/+}$ and Trf1$^{-/-}$ MEFs were treated with Cre and 2 days after selection synchronised in G1/S. The indicated proteins were analysed by immunoblotting. Quantification of indicated proteins relative to actin. Statistical significance determined by two-way ANOVA. p values indicated. n is the number of individual MEFs per genotype. **(e-f)** Representative immunofluorescence images of *Trf1*$^{+/+}$ and *Trf1*$^{-/-}$ MEFs stained with antibodies against TRF1 (green) and Aurora B (red) showing no alteration in the localization of Aurora B in the absence of TRF1. Bars = 7.5μM **(f)** Representative images of immunofluorescence images of Trf1$^{+/+}$ and Trf1$^{-/-}$ MEFs stained with Aurora B (red) and α-Tubulin (green) to emphasise the correct localization of Aurora B in cells lacking TRF1.

Supplemantary Figure 4 (a) Representative time-lapse images of *wild-type* and *G3 Terc*$^{-/-}$. Selected time points are shown. Cells are transfected with H2B-CHERRY (red) to stain DNA. **(b)** Quantification of the time taken to complete mitosis (left panel) and the percentage of cells that complete cytokinesis (right panel) of 3 independent *wild-type and G3 Terc*$^{-/-}$ MEFs. Error bars represent standard error. Student's t test or ANOVA were used for statistical analysis and p values are indicated.