Gu et al. Supplementary Figures

Supplementary Figure 1. *CTC1* knockout construct. (A) The various CTC1 alleles are indicated schematically. Black boxes, coding exons, white box, non-coding exon; red box, exon 6; black arrowheads, PCR genotyping probes; blue arrowheads, loxP sites; blue rectangle, PGK-neo gene; EV, EcoRV, sizes of DNA fragments are shown; blue rectangles, left and right arm probes for genomic Southern. (B) The CTC1 gene has 23 coding exons. Deletion of exon 6 generates a frameshift and a protein product of only 234 amino acids which does not contain any of the 4 OB folds present in the original protein. Met, initiating methionine; arrowheads, PCR genotyping primers. (C) Southern analysis of genomic DNA digested with EcoRV of 4 independent ES cell lines with the 5’ and 3’ probes. (D) RT-PCR genotyping with the indicated primers labeled in B reveal efficient deletion of the *CTC1* floxed allele in *CAG-CTC1F/F* MEFs. GAPDH primers were used as RNA control. V, vector.

Supplementary Figure 2. Consequences of CTC1 deletion. (A) Immunoblot demonstrating STN1 levels in WT and CTC1 null MEFs. γ-tubulin served as loading control. (B) Telomere-FISH analysis of vector treated *CTC1*−/− MEFs, and WT MEFs treated with either vector, or shRNAs against CTC1 or STN1, for the presence of endogenous STN1 at telomeres. Arrowheads, STN1 localized to telomeres. (C) CTC1 null mice are smaller than their WT littermates. (D) Diminished STN1 protein levels in CTC1 null tissues. γ-tubulin served as loading control.

Supplementary Figure 3. Organ sizes in CTC1 null mice. (A) Individual and average weights of thymus (P=0.0195) (B) kidney (P=0.0141) and (C) testes (P=0.236) from WT and *CTC1* null mice are indicated.

Supplementary Figure 4. *CTC1* deletion induces proliferative arrest. (A) Top, C-kit/Sca-1 FACS analysis of fetal livers isolated from E15.5 day old *CTC1* null embryos display decreased LK cells. Bottom, BrdU/7-ADD cell cycle analysis of WT and *CTC1* null fetal livers. (B) Summary of *in vivo* BrdU labeling of WT and CTC1 null mice. Percent of labeled cells in testis, small intestine and skin are indicated.
Supplementary Figure 5. Deletion of CTC1 results in increased single-stranded G-overhang. In-gel hybridization analysis of genomic DNA isolated from spleen (A), bone marrow (B) and MEF (C) of CTC1+/+ and CTC1−/− mice or MEF under native (middle panels) and denaturation conditions (bottom panels) with a 32P-labeled [CCCTAA]4-oligo probe to detect ss (top) and total (bottom) telomere DNA. Overhang signal intensity (%) was normalized to total telomeric signals. 10 µg of total DNA from tissues or cells was loaded in each lane. Ethidium bromide staining (top panels) served as loading controls. Molecular weight markers are indicated.

Supplementary Figure 6. Deletion of CTC1 does not cause increased single-stranded G-overhang in quiescent tissue liver. (A) In-gel hybridization analysis of genomic DNA isolated from liver of CTC1+/+ and CTC1−/− mice under native (left panel) and after denaturation (right panel) with a 32P-labeled [CCCTAA]4-oligo probe to detect ss (top) and total (bottom) telomere DNA. Pot1bΔ/Δ liver was used as a positive control in the last lane. (B) Quantification of (A). Overhang signal intensity (%) was normalized to the total telomeric signals.

Supplementary Figure 7. Acute deletion of CTC1 in CAG-CreER−CTC1F/F MEF with 4-hydroxy-tamoxifen (4-HT) causes telomere loss. (A) Immunoblot demonstrating STN1 levels in CAG-CreER; CAG-CreER, CTC1F/F (2 independent lines) and CTC1F/F MEFs treated with 4-HT. γ-tubulin was served as loading control. (B) & (C) Quantification of mRNA level of CTC1 (B) and STN1 (C) by RT-PCR after treatment with 4-HT. (D) Quantification of telomere-free chromosome ends after treatment of 4-HT for 21 days. Error bars: s.e.m. (E), (F) Quantification of mRNA level of STN1 (E) and CTC1 (F) in wild type MEFs treated with shRNAs against STN1 and/or CTC1, respectively.

Supplementary Figure 8. (A) Telomere-PNA FISH demonstrating that localization of the shelterin components on telomeres are not affected upon CTC1 deletion. Wild type MEF, CTC1−/− MEF, wild type MEF treated shSTN1 and CTC1−/− MEF reconstituted with CTC1 cDNA were stained with the indicated antibodies against TRF1, TRF2, RAP1 and TPP1 (green), telomere-PNA FISH (red) and DAPI (blue). (B) Quantitation of (A) with the TIF>10 per nuclei. At least 50 cells were counted.
Supplementary Figure 9. *CTC1* depletion hinder telomere synthesis and reduce efficient restart of stalled replication at telomeres. (A) FACS analysis of DNA content in synchronized MEFs with conditional Ctc1 deletion. (B) Representative slot blot of separated leading/lagging/unreplicated telomeres from synchronized MEFs labeled with BrdU. After centrifugation, fractions were collected from bottom of the gradient (high density) to top (low density), DNA in each fraction was loaded on membrane from left to right, denatured, and then hybridized with G-rich telomeric probe. (C) Differences of BrdU incorporation into leading and lagging strand telomeres, and amount of unreplicated total telomeres, after treatment of *CTC1FF* and *CAG-CreER; CTC1F/F* MEFs with 4-hydroxytamoxifen (4-HT). Mean values were derived from 4 individual experiments. Calculations were performed as follows: BrdU incorporation (no treatment) – BrdU incorporation (after 4-HT)/BrdU incorporation (no treatment). Error bars: s.e.m. (D) Schematic of experimental design for measuring the efficiency of the restart of stalled replication at telomeres.
Gu et al., Supplementary Figure 1
**A**

WT and CTC1–/–

STN1

γ-Tubulin

**B**

STN1, Telo, merge

WT + Vector

WT + shStn1

WT + shCTC1

CTC1–/–

**C**

2171 WT

2169 WT

2173 CTC1–/–

2174 CTC1–/–

24 day old

**D**

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Gu et al., Supplementary Figure 2
A

Thymus
P=0.0195

ratio to body weight (%)

WT
CTC1-/-

WT
CTC1-/-

B

Kidney
P=0.0141

ratio to body weight (%)

WT
CTC1-/-

WT
CTC1-/-

C

Testes
p=0.236

ratio of body weight

WT
CTC1-/-

WT
CTC1-/-

Gu et al., Supplementary Figure 3
A

WT, n=2

CTC1⁻/⁻, n=2

B

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Gu et al., Supplementary Figure 4
Gu et al., Supplementary Figure 5
Gu et al., Supplementary Figure 6
A

Wild type MEF

CTC1⁻/⁻ MEF

shSTN1 MEF

CTC1⁻/⁻ MEF + Flag-CTC1

B

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Gu et al., Supplementary Figure 8
Gu et al., Supplementary Figure 9