Supporting Information

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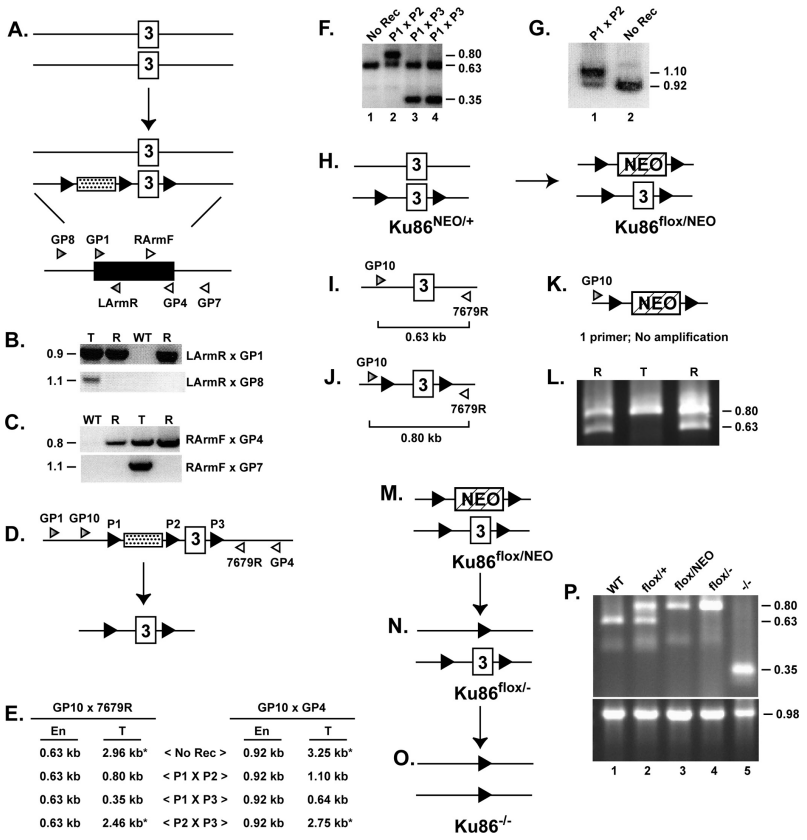


Fig. S1. Molecular evidence for the construction of a conditionally null Ku86 human cell line. (*A*) Cartoon of the diploid wild-type Ku86 locus with exon 3 highlighted. In the first step, 1 allele is modified by using a targeting vector containing 3 LoxP sites (black triangles). The NEO gene is cartooned as a stippled rectangle. A blowup of the targeted allele is shown below, and the relevant region is simplified as a filled rectangle. The location of relevant PCR primers is shown. The primers used for the diagnostic 5′ PCR are shown as gray triangles, and the primers used for the diagnostic 3′-PCR are shown as open triangles. Importantly, GP8 and GP7 sequences reside outside of the targeting vector in the flanking genomic DNA and can therefore be used to identify correctly targeted integration events. (*B*) The diagnostic 5′ PCR band from LArmR × GP8 is seen in the Ku86$^{NEO/+}$ cell line (T), but not in 2 randomly (R) targeted colonies, nor in the parental (WT) cells. An ethidium bromide (EtdBr)-stained agarose gel is shown. (*C*) The diagnostic 3′ PCR band from a RArmF × GP7 PCR is seen in the Ku86$^{NEO/+}$ (T) cell line but not in 2 R colonies, nor in the WT cells. An EtdBr-stained agarose gel is shown. (*D*) The Ku86$^{NEO/+}$ cell line was then treated with a Cre expression plasmid, and cells (Ku86$^{flox/+}$) that had undergone the Cre-mediated LoxP recombinational loss of NEO, but not exon 3, were identified. The 3 LoxP sites are now designated P1, P2, and P3, respectively, to help clarify the PCR strategy used in the identification. (*E*) The relevant PCR reactions are listed, as are the expected sizes of the PCR products for the endogenous (En) unmodified allele and for the targeted (T) allele. The PCR extension times were sufficiently short, such that the longer PCR products, which are designated with asterisks, were not generated. The desired product was a clone of cells that had undergone a P1 × P2 recombination event, and these needed to be distinguished from P1 × P3 events. Cell not undergoing recombination (No Rec) and P2 × P3 recombination events were not distinguished from one another, but they could be identified by the retention of the NEO gene and the accompanying G418 resistance. (*F*) The 0.8-kb band diagnostic of a P1 × P2 recombination from a GP10 × 7679R PCR is seen in the P1 × P2 lane (lane 2) but not in 2 P1 × P3 colonies (lanes 3 and 4), nor in the No Rec (lane 1) cells. An EtdBr-stained agarose gel is shown, and the molecular weights of the PCR products are indicated. (*G*) The 1.10-kb band diagnostic of a P1 × P2 recombination from a GP10 × GP4 PCR is seen in the P1 × P2 lane (lane 1) but not in the No Rec (lane 2) cells. An EtdBr-stained agarose gel is shown, and the molecular weights of the PCR products are indicated. (*H*) The cell line containing 1 floxed Ku86 exon 3 (Ku86$^{flox/+}$) was then subjected to a second round of targeting in which the desired product was a cell line where the remaining unmodified allele was replaced by the NEO cassette. Specifically, G418-resistant clones were first selected, and then clones that had lost the diagnostic 0.63-kb band (*I*) but retained the 0.8-kb band representing the floxed allele (*J*) were identified. Importantly, the Ku86 exon 3 knockout vector lacked the sequences corresponding to 7679R (*K*), and so it was not amplified in this screen. (*L*) One correctly targeted clone (T) corresponding to Ku86$^{flox/NEO}$ cells was identified from a large number of randomly (R) targeted clones. An EtdBr-stained agarose gel is shown, and the molecular weights of the relevant PCR products are indicated. The Ku86$^{flox/NEO}$ cells (*M*) were then exposed to Cre recombinase, and live, G418-sensitive cells (Ku86$^{flox/-}$) were selected. These ''conditionally null'' cells (N) are viable functional heterozygotes, and they could be expanded and propagated as desired. When needed, the Ku86$^{flox/-}$ cells were once again exposed to Cre recombinase to generate the null (Ku86$^{-/-}$) cell line (*O*). (*P*) A final diagnostic PCR using GP10 × 7679R primers (*E*) was performed to confirm the identity of WT (lane 1), Ku86$^{flox/+}$ (lane 2), Ku86$^{flox/NEO}$ (lane 3), Ku86$^{flox/-}$ (lane 4), and Ku86$^{-/-}$ (lane 5) cell lines (*Upper*). A control PCR using flanking intron 2 primers (GP1 and GP2) was used as a loading control (*Lower*). EtdBr-stained agarose gels are shown, and the molecular weights of the relevant PCR products are indicated.

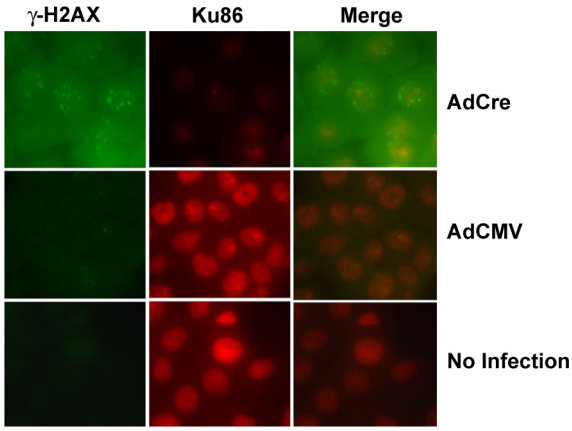
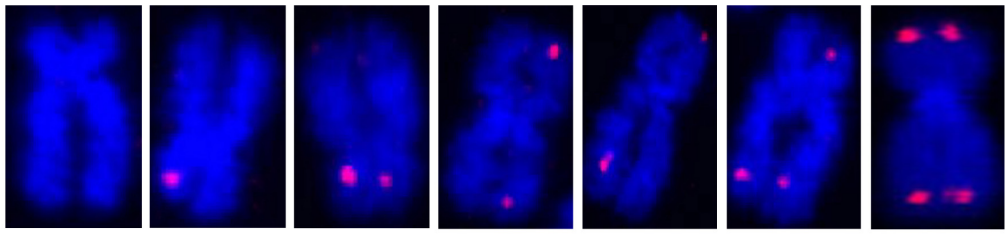


Fig. S2. DNA DSBs correlate with the absence of Ku86. Ku86$^{flox/-}$ cells were either left untreated (No Infection) or were infected with AdCre or AdCMV. Cells were fixed 5 days later and stained with fluorescently tagged secondary antibodies directed against primary antibodies for γ-H2AX (green) or Ku86 (red), and then the panels were overlaid (merge). (Magnification: 100×.)



Metaphase:					*			Totals:
#1	15	17	3	4	0	4	1	44
#2	6	13	6	4	2	3	4	38
#3	13	13	6	1	3	5	1	42
#4	12	13	9	1	1	3	1	40
#5	19	17	4	2	0	0	0	42
#6	15	10	2	3	2	6	1	39
#7	19	13	2	6	1	1	0	42
#3	13	9	9	2	2	5	0	40
#9	5	15	5	1	2	7	5	40
Totals:	117	120	46	24	13	34	13	367
Percentage:	31.9%	32.7%	12.5%	6.5%	3.5%	9.3%	3.5%	

Fig. S3. Ku86 null cells randomly lose their leading- or lagging-strand telomeres. Nine individual metaphases were scored for whether they had lost 1, 2, 3, or 4 of their telomeric FISH signals in the configuration indicated by the figures. Chromosomes containing only 2 FISH signals can retain them in 3 potential configurations, and those metaphases where the 2 signals are diagonally juxtaposed on adjacent sisters (marked by an asterisk) would be indicative of specifically leading- or lagging-strand telomere loss. These metaphases were, however, less abundant then the other 2 configurations, which indicated that the leading- and lagging-strand telomeres were lost randomly. Note that the total number of chromosomes scored per metaphase was always less than the theoretical maximum of 46, not because chromosomes were lost (which was almost never seen) but because only chromosomes where all 4 arms could be unambiguously scored were included in the analysis. (Magnification: 120×.)

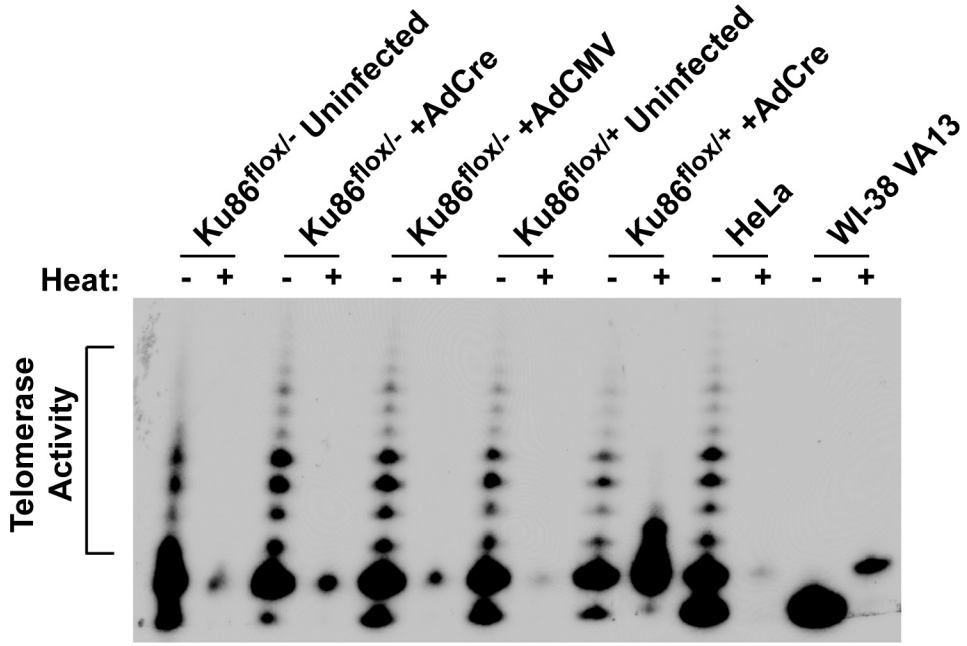


Fig. S4. Ku86 null cells still contain telomerase activity. Cellular extracts were prepared from the indicated Ku86-derivative cell lines. Before use, the extracts were either left untreated (−) or heated (+) to inactivate telomerase. The extracts were then used in a TRAP assay. All of the Ku86-derivative HCT116 cell lines contained robust telomerase activity. As controls, extracts were prepared from HeLa cells, which are known to be telomerase-positive, as well as from WI-38 VA13 cells, which are known to be telomerase-negative.

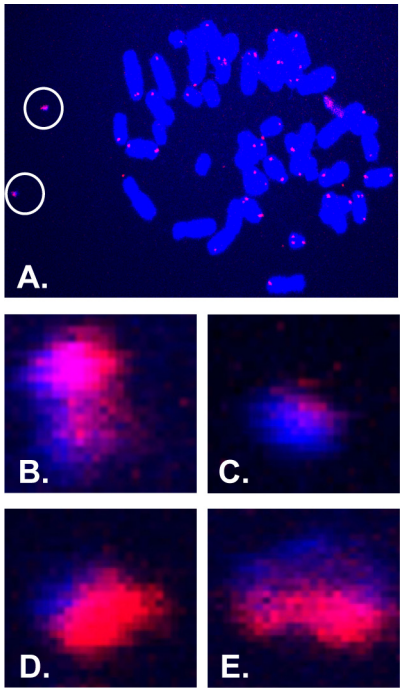


Fig. S5. Human Ku86 null cells contain extrachromosomal FISH signals. FISH analyses of metaphase chromosomes with a telomere-specific Cy3-$(C_3TA_2)_3$ protein–nucleic acid probe. Telomeres are seen as red dots, and metaphase chromosomes are stained blue. (*A*) A complete metaphase showing the existence of extrachromosomal FISH signals (white circles). (*B–E*) Four independent examples of extrachromosomal FISH signals demonstrating that they costain with DAPI. (Magnification: 120×.)

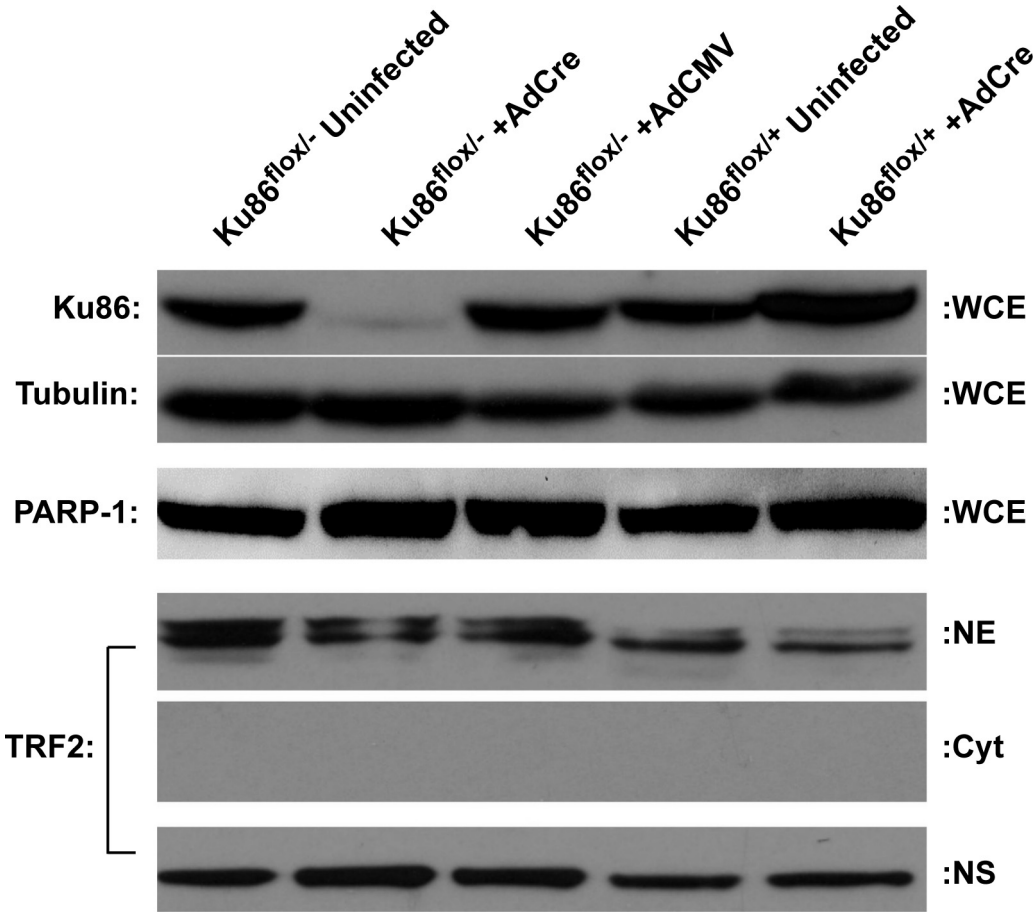


Fig. S6. Ku86 null cells do not undergo apoptosis and do not show alterations in the level or localization of TRF2. Cellular extracts, either whole-cell (WCE), nuclear (NE), or cytoplasmic (Cyt), were prepared from the indicated Ku86-derivative cell lines. The extracts were then subjected to Western blot analyses using the indicated antibodies. The Ku86 Western blot demonstrated that the $Ku86^{flox/-}$ +AdCre cells lacked Ku86 protein. The tubulin Western blot was used as a loading control. The PARP-1 Western blot demonstrated that Ku86 null cells were not apoptotic, and the TRF2 immunoblots demonstrated that the levels of TRF2 were not significantly altered and that the protein was in the expected cellular compartment. NS, nonspecific band used as an NE loading control.

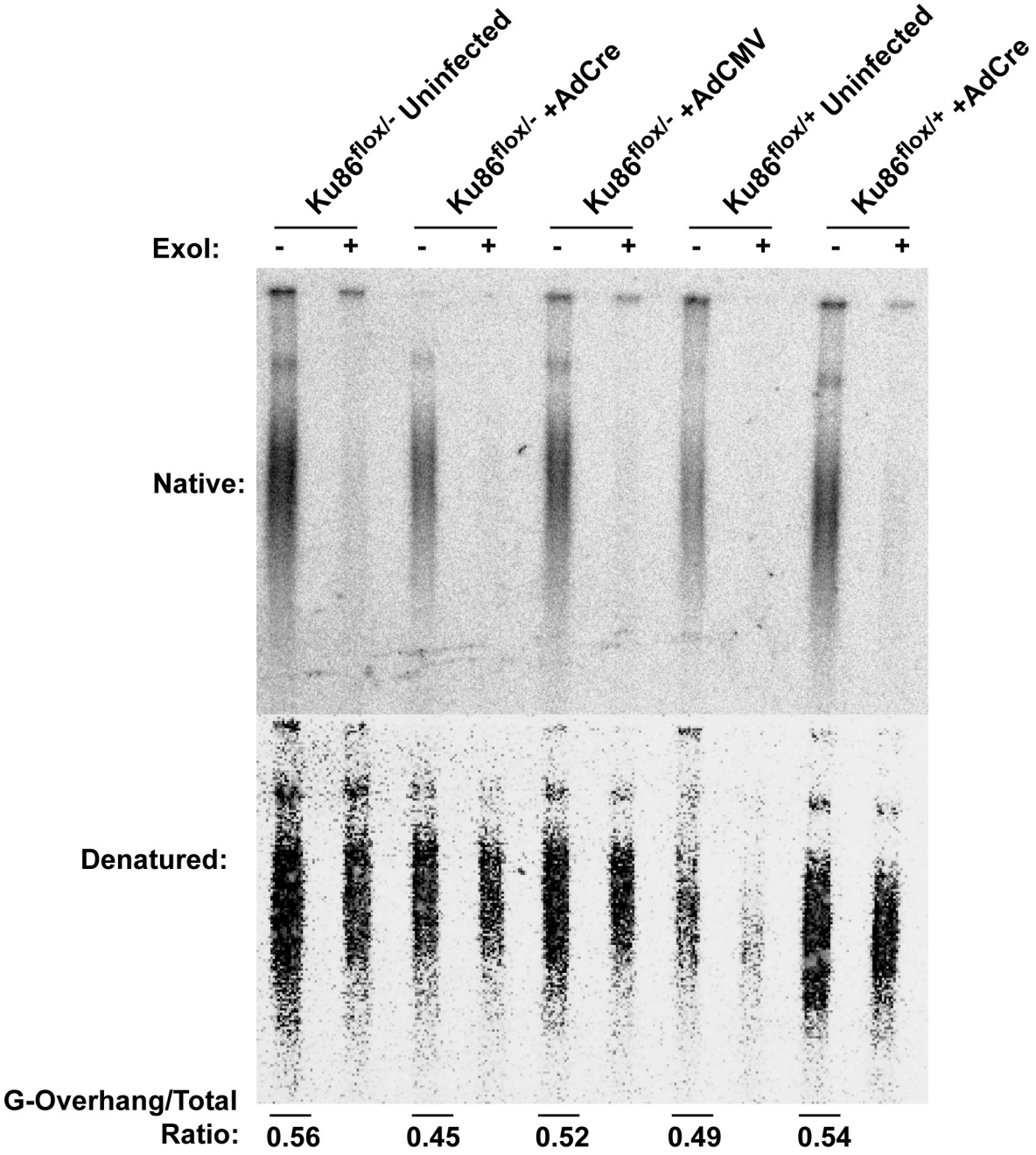


Fig. S7. The G overhang is not altered in Ku86 null cells. Genomic DNA was purified from the indicated cell lines and either left untreated (−) or digested overnight (+) with ExoI. The DNA was subsequently purified, digested to completion with MboI and AluI, and then subjected to terminal restriction fragment Southern blot analysis under nondenaturing (Native) conditions with a $(C_3TA_2)_3$ 5′ end-radiolabeled oligonucleotide probe. The gel was subsequently denatured and rehybridized with the identical probe (Denatured). Autoradiograms of the gels were quantitated, and the ratio of signal derived from a sample on the Native gel versus the signal from the Denatured gel is presented below the blots as the G-Overhang/Total Ratio. There was no alteration of the G overhang in Ku86 null cells.