Perifosine as a potential novel anti-telomerase therapy

Supplementry Material

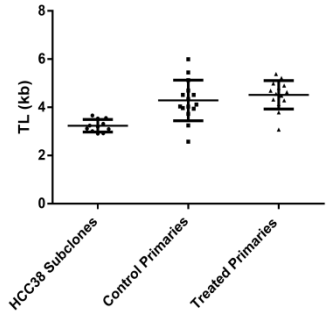

Figure S1: telomere length in primary tumors compared with subclones of HCC38. Mean telomere length in both control and treated primary tumors was longer than subclones of the parental HCC38+LUC+GFP population, suggesting that the difference in telomere length between the parent population and the primary tumors was not due to a founder effect.

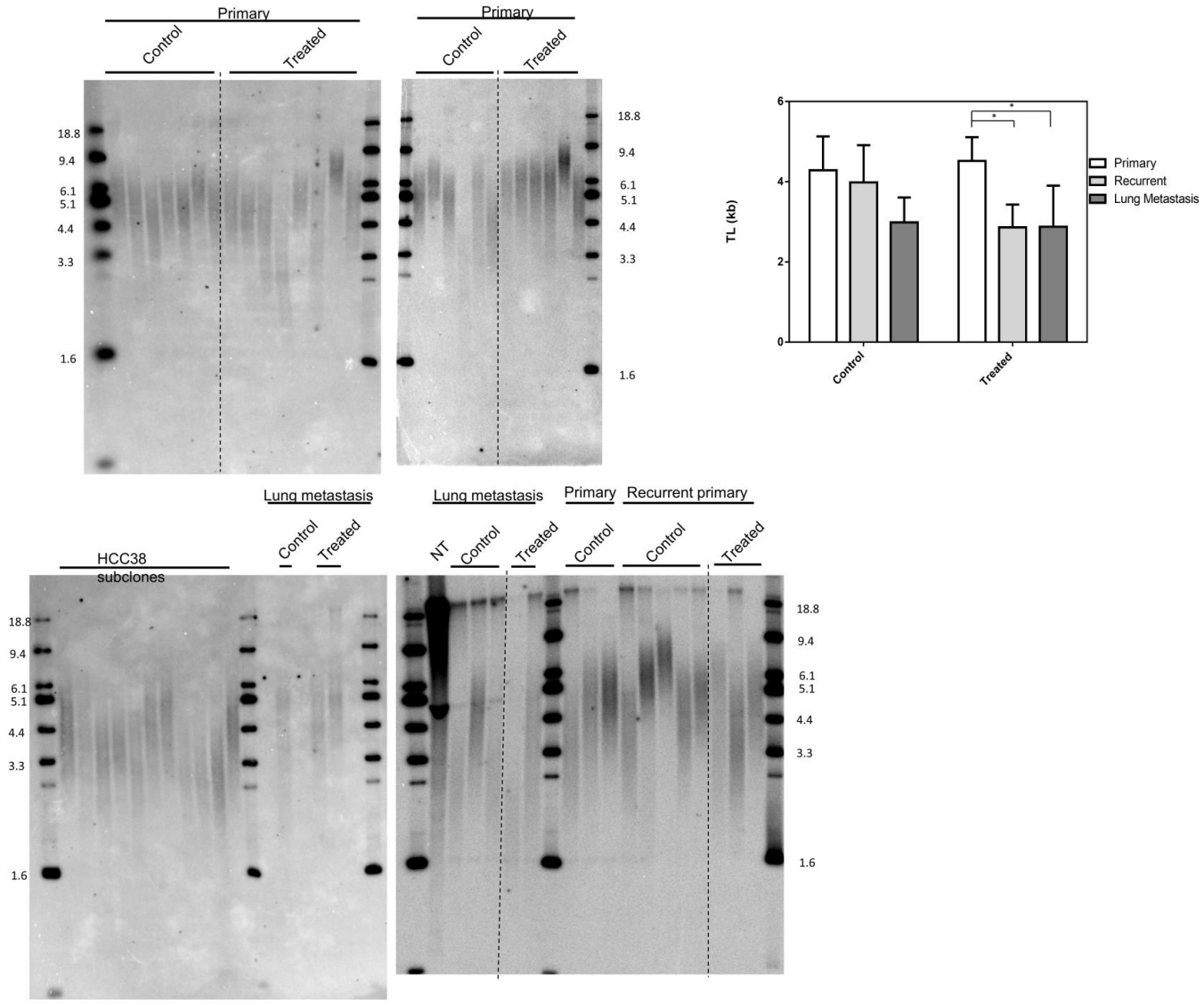

Figure S2: Terminal Restriction Fragment (TRF) primary data for figure 2. Mean telomere length was computed as described in [37]; the band indicative of mouse cell contamination is clearly visible in telomeric DNA extracted from a non-tumor-bearing mouse lung (NT, middle right gel).

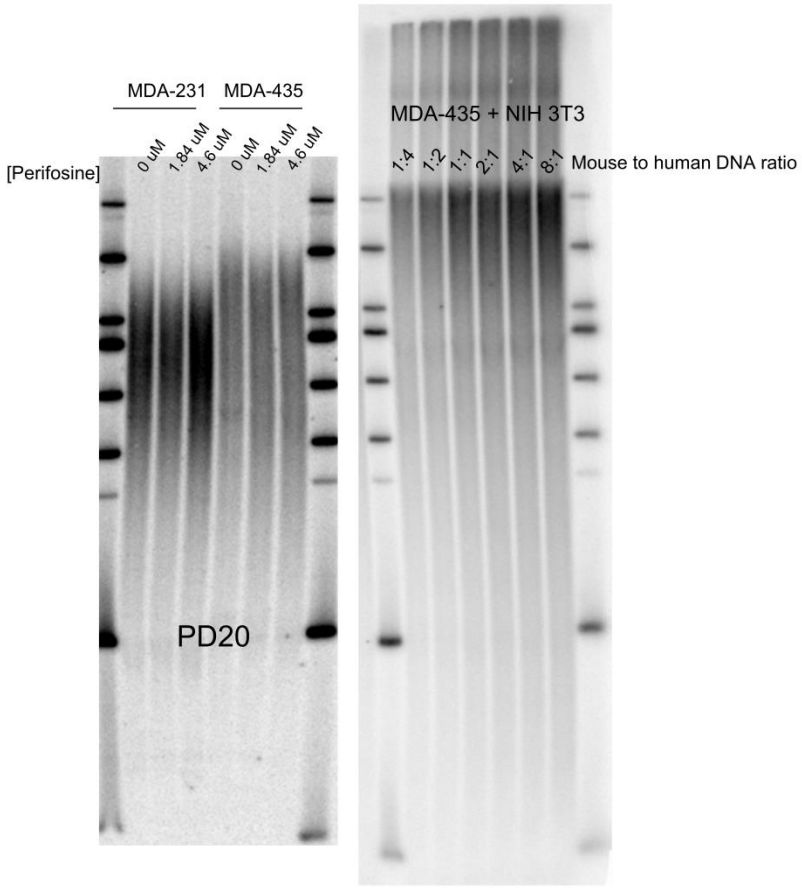

Figure S3: Small quantities of murine DNA can dramatically bias measurement cancer cell telomere length. Varying ratios of DNA purified from mouse NIH 3T3 cells to human MDA-MB-435 cells DNA were mixed prior to TRF digestion and analysis. Small quantities of mouse DNA (20% by mass, a ratio of 1:4 mouse to human) were sufficient to bias measurement of MDA-MB-435 cells (right panel) compared with measurement of MDA-MB-435 cells alone (left panel).

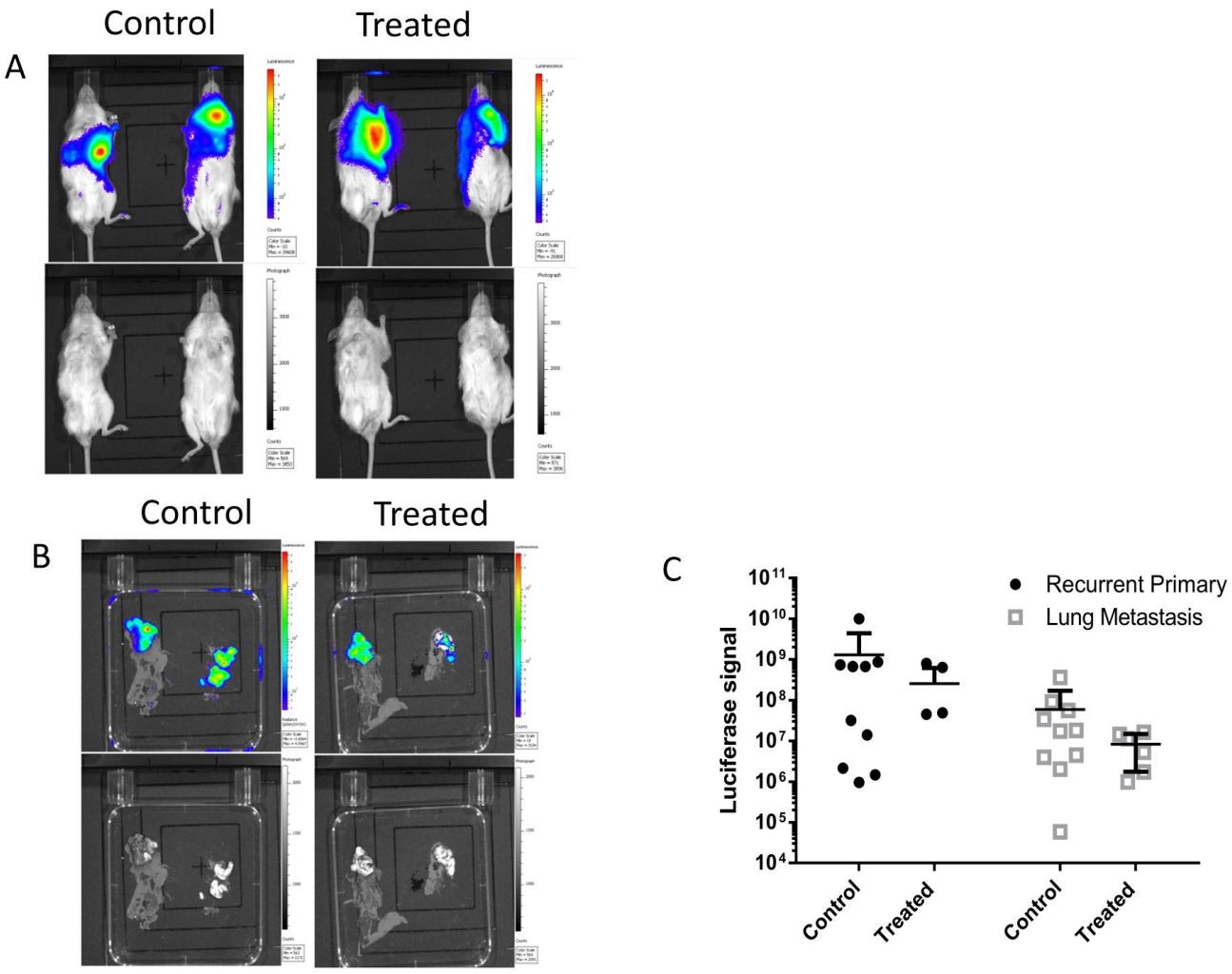

Figure S4: Representative pictures of bioluminescence signal from recurrent primary and lung metastatic tumors. There was no statistically significant difference in tumor burden between control and Perifosine-treated mice as measured by bioluminescence signal in either recurrent primary (A) or metastatic tumors (B).

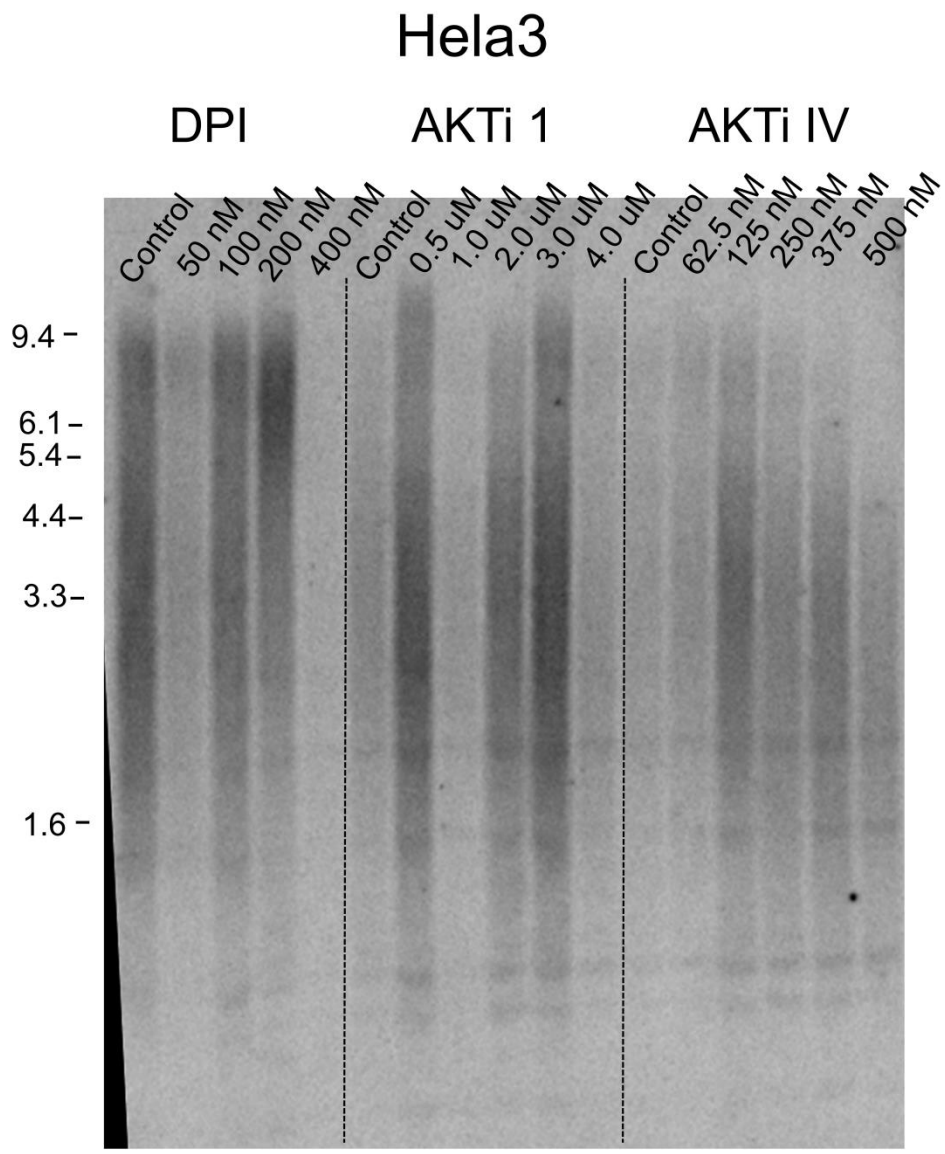

Figure S5: Tests for telomere shortening in Hela cells exposed to other AKT inhibitors. Hela cells treated with sub-lethal concentrations of AKT inhibitor IV exhibit telomere shortening over time, while cells treated with AKT inhibitor I and the Dual Pathway Inhibitor did not exhibit telomere shortening.

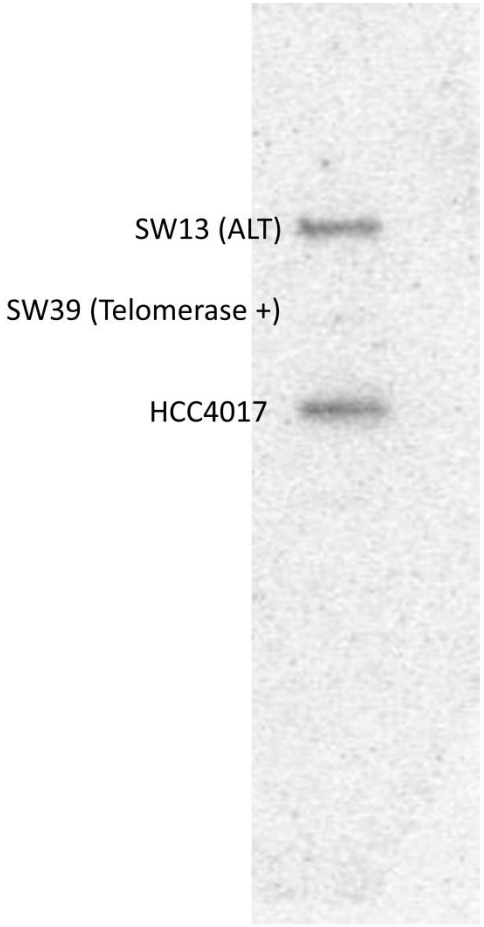

Figure S6: C-circle assay on HCC4017. HCC4017 exhibited a number of c-circles consistent with document Alternative Lengthening of Telomeres (ALT)-utilizing cell lines.

Table S1: Summary of *in vitro* data. A summary of the cells lines evaluated for changes in telomere length between untreated and the highest measured dose of Perifosine for each cell line (middle) as well as a summary of the change in telomerase enzymatic activity in each cell line (right column).

Cell Line	Telomere Length	Telomerase activity
HCC4017	Unchanged	Increased
H2087	Lengthening	Increased
HCC827	Unchanged	Decreased
H2009	Shortening	Unchanged
H2882	Shortening	Decreased
HCC15	Shortening	Increased
H1819	Shortening	Unchanged
HCC515	Unchanged	Unchanged
HCC2429	Shortening	Decreased
Hela	Shortening	Decreased
HT1080	Lengthening	N/A
H1993	Shortening	N/A
H2073	Shortening	N/A
HCC95	Unchanged	N/A
MDA-MB-231	Shortening	N/A
MDA-MB-435	Shortening	N/A
DLD1	Shortening	N/A
A375	Unchanged	N/A
HCC38	Shortening	N/A
H1299	Unchanged	N/A