Supplementary Information:

Selective Elimination of Osteosarcoma Cell Lines with Short Telomeres by Ataxia Telangiectasia and Rad3-Related Inhibitors

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Table S-1 – Origin and Characteristics of the Osteosarcoma Cell Lines

Cell Line	Media	Age (Years)[a]	Sex[a]	Reference[b]
HOS-MNNG[c]	RPMI	13	Female	ATCC-CRL-1547
OHSN	RPMI	14	Male	*Fodstad et al. 1986* [1]
SJSA-1	RPMI	19	Male	ATCC-CRL-2098
HAL	RPMI	16	Male	ExPASy (CVCL_D788)
143b[c]	DMEM	13	Female	ATCC-CRL-8303
HOS	EMEM	13	Female	ATCC-CRL-1543
MG-63	EMEM	14	Male	ATCC-CRL-1427
MHM	RPMI	41	Female	*Kjønniksen et al. 1994* [2]
HuO-3N1	RPMI	15	Female	ExPASy (CVCL_1297)
G292	McCoy's	9	Female	ATCC-CRL-1423
HuO-9	RPMI	13	Female	ExPASy (CVCL_1298)
CAL72	DMEM	10	Male	ExPASy (CVCL_1113)
U2OS	McCoy's	15	Female	ATCC-HTB-96
KPD	RPMI	7	Female	*Bruland et al. 1988* [3]
NY	RPMI	15	Male	ExPASy (CVCL_1613)
SAOS-2	DMEM	11	Female	ATCC-HTB-85
LM7[d]	DMEM	11	Female	*Jia et al. 1999* [4]

[a] Age and sex of the patient from whom the tumour originates
[b] Identifiers given for cell lines available at the ATCC or ExPASy
[c] metastatic derivatives of HOS; [d] metastatic derivative of SAOS-2

Table S-2 – Telomere length measurement from TRF in Figure 1c

Cell Line	Telomere status	Mean Length (kb)	Median Length (kb)	Variance (kb^2)	Semi-interquartile range (kb)
HEK293T	Control	6.64	5.74	16.72	3.64
HOS-MNNG	ST	5.21	2.98	20.44	1.86
OHSN	ST	5.02	3.22	19.12	2.02
SJSA	ST	4.83	3.21	17.93	2.12
HAL	ST	4.71	2.70	22.74	1.90
143b	ST	4.42	2.96	16.21	2.02
HOS	ST	4.70	3.58	16.73	2.36
MG-63	LT	6.78	5.77	12.29	4.46
MHM	LT	6.73	6.37	12.94	4.18
HuO-3N1	LT	6.06	6.67	20.63	1.76
G292	ALT	21.54	10.69	524.47	6.36
HuO-9	ALT	17.27	9.67	348.21	5.72
CAL72	ALT	20.24	10.24	477.35	6.22
U2OS	ALT	25.21	13.72	577.77	7.34
KPD	ALT	18.95	9.95	392.04	5.94
NY	ALT	19.19	9.89	390.53	5.84
SAOS-2	ALT	13.81	7.61	251.98	4.62
LM7	ALT	15.56	8.20	293.43	4.90

ST – ALT-negative, short telomere
LT – ALT-negative, long telomere
ALT – ALT-positive

Table S-3 – IC50 drug response in osteosarcoma cell lines

Cell Line	Telomere status	AZD-6738 µM	VE-822 µM	BEY-1895344 µM	Methotrexate µM
HOS-MNNG	ST	0.29±0.02	0.12	0.017±0.001	0.04
OHSN	ST	0.66±0.18	0.25±0.06	0.013±0.001	0.20
SJSA	ST	22.68±3.49	1.98±0.18	0.537±0.128	0.30
HAL	ST	2.26±0.42	0.69±0.01	0.081±0.003	0.07
143b	ST	0.69±0.14	0.09	0.018	0.02
HOS	ST	0.83±0.48	0.29±0.06	0.014±0.005	0.32
MG-63	LT	3.97±0.19	1.30	0.061	197.8
MHM	LT	2.20±0.45	0.36±0.05	0.044±0.002	67.87
HuO-3N1	LT	9.56±3.98	3.06±1.85	0.233±0.154	71.85
G292	ALT	3.16±2.66	1.30±1.12	0.152±0.070	69.11
HuO-9	ALT	1.08±0.57	0.75	0.024	0.06
CAL72	ALT	0.80±0.03	0.13	0.015±0.002	44.30
U2OS	ALT	4.91±2.16	1.44±0.18	0.066±0.016	0.03
KPD	ALT	2.01±1.70	0.18	0.678	57.84
NY	ALT	2.54±1.34	1.68	0.059	0.09
SAOS-2	ALT	5.17±1.29	1.58	0.206	0.01
LM7	ALT	9.92±0.71	1.80±0.49	0.574±0.365	0.60

Table S-4 – Oligonucleotides used for this study

Oligo name	Sequence
hTeloG	ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT
hTeloC	TGTTAGGTATCCCTATCCCTATCCCTATCCCTAACA
AlbuminF	CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG
AlbuminR	GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT
GlobinF	CGGCGGCGGGCGGCGCGGGCTGGGCGGCTTCATCCACGTTCACCTTG
GlobinR	GCCCGGCCCGCCGCGCCCGTCCCGCCGGAGGAGAAGTCTGCCGTT
CC-TeloF	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
CC-TeloR	TCCCGACTATCCCTATCCCTATCCCTATCCCTA
hTERT F1579	GCTGACGTGGAAGATGAGCGTGC
hTERT R1616	TCCTCACGCAGACGGTGCTCTG
hTERC F27	GGTGGTGGCCATTTTTTGTC
hTERC R163	GTAGAATGAACGGTGGAAG
7SK F7	GAGGGCGATCTGGCTGCGACAT
7SK R112	ACATGGAGCGGTGAGGGAGGAA
GAPDH F6	GAAGGTGAAGGTCGGAGT
GAPDH R231	GAAGATGGTGATGGGATTTC
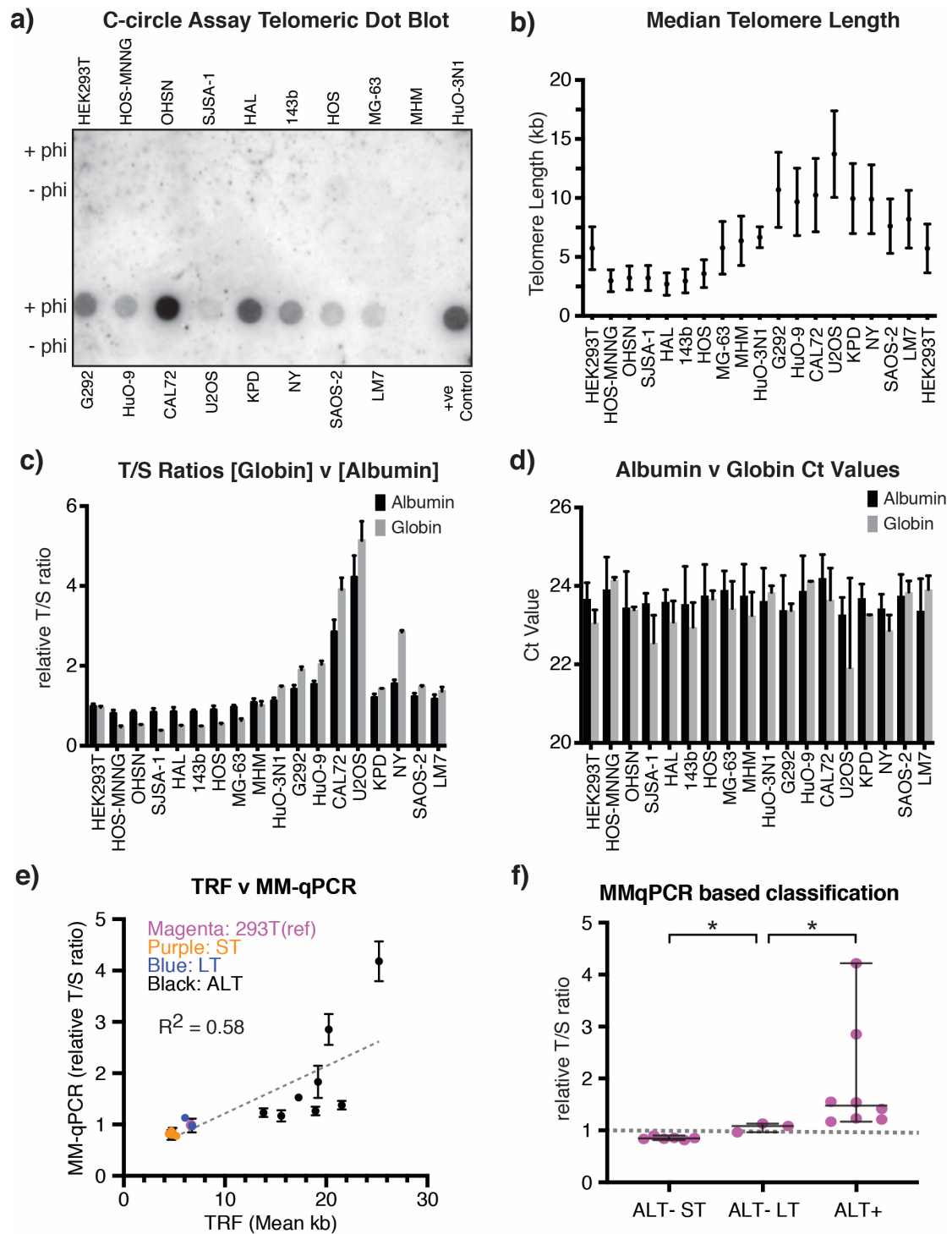
Figure S-1 – Characterisation of Telomere Status in Osteosarcoma Cell Lines

a) *Dot Blot from C-Circle Assay.* C-circle amplification products were detected by dot blot using a telomeric TTAGGG probe. The pCR4 plasmid containing telomeric repeats was used as a positive control. Phi indicates Phi-29 polymerase.

b) *Box plot of median telomere lengths from TRF analysis in Figure 1c as calculated by Telometric software.* Graph shows the median and interquartile range of the telomere fragments.

c) *Comparison of T/S ratios obtained using albumin or beta-globin as the single copy gene (SCG).* T/S ratio is expressed relative to the T/S ratio determined for HEK293T cell

line as expressed as the fold enrichment. Data represent the mean of three independent experiments, each MM-qPCR run in triplicate. Error bars show standard deviation.

d) Uniformity of Ct (cycle threshold) values obtained with *albumin* locus, compared to the *beta-globin* locus. Standard deviation, standard error of the mean and coefficient of variation for the *albumin* and *beta-globin* loci were 0.234 v 0.568, 0.055 v 0.134 and 0.99% v 2.43%, respectively).

e) Correlation graph between TRF-based and MM-qPCR-based telomere length measurements. T/S ratio was determined using *albumin* gene as a SCG, and are expressed relative to that in HEK293T cell line. Liner regression is indicated as grey dashed Line. TMM status of the samples and reference HEK293T are colour coded as indicated.

f) MM-qPCR derived telomere repeats variation in OS cell lines grouped according to TMM status. The grey dashed line indicates the T/S ratio of HEK293T. Mann-Whitney U Test indicates $p=0.0238$ and $p=0.0121$ comparing ALT -ST vs ALT-LT and ALT+ vs ALT-LT, respectively.
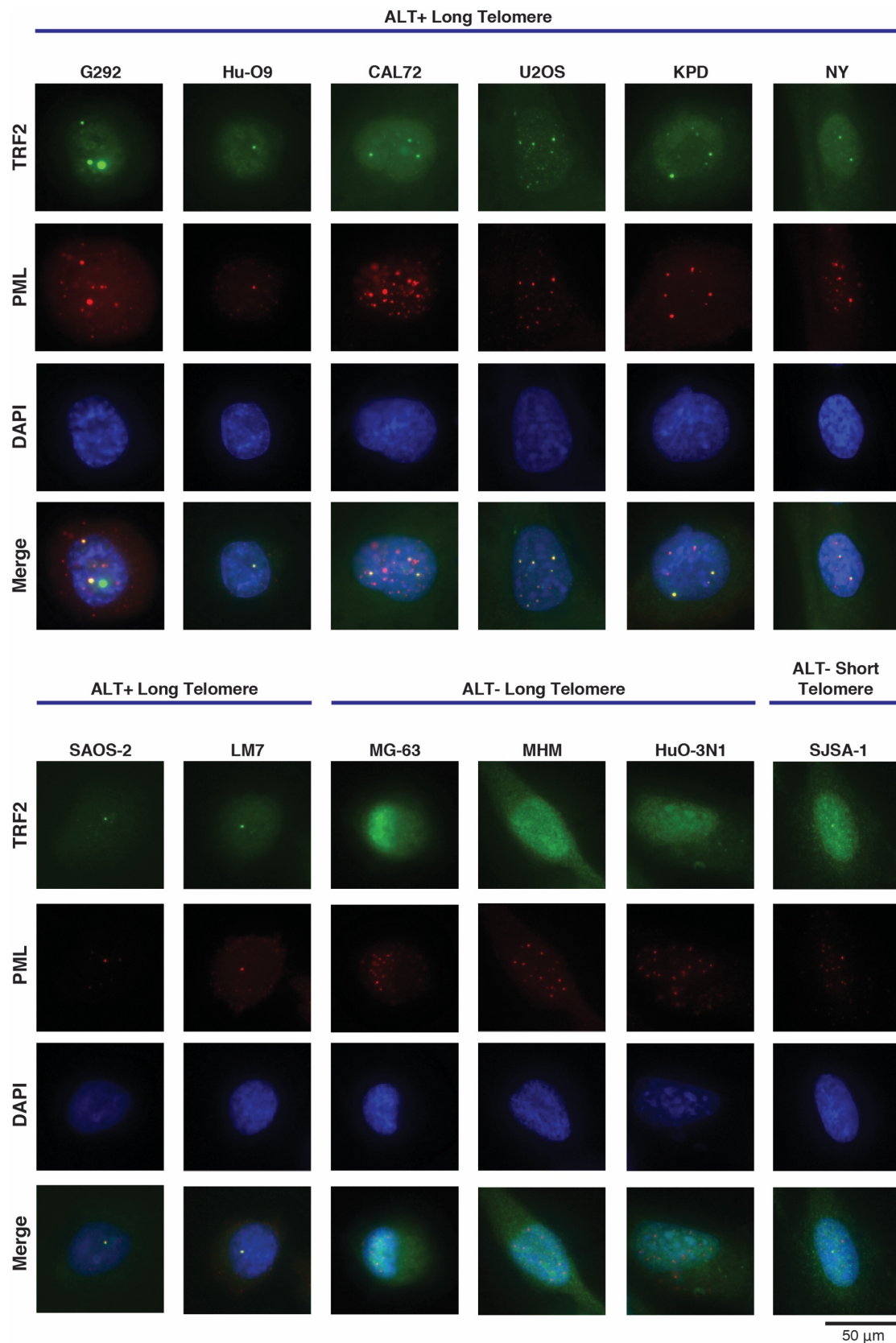
Figure S-2 – APB Assay
Representative immunofluorescence images showing the presence/absence of ALT associated PML bodies (APBs) in the osteosarcoma cell lines with long telomeres. SJSA-1 is shown as a negative control. A 50-μm scale bar is shown.
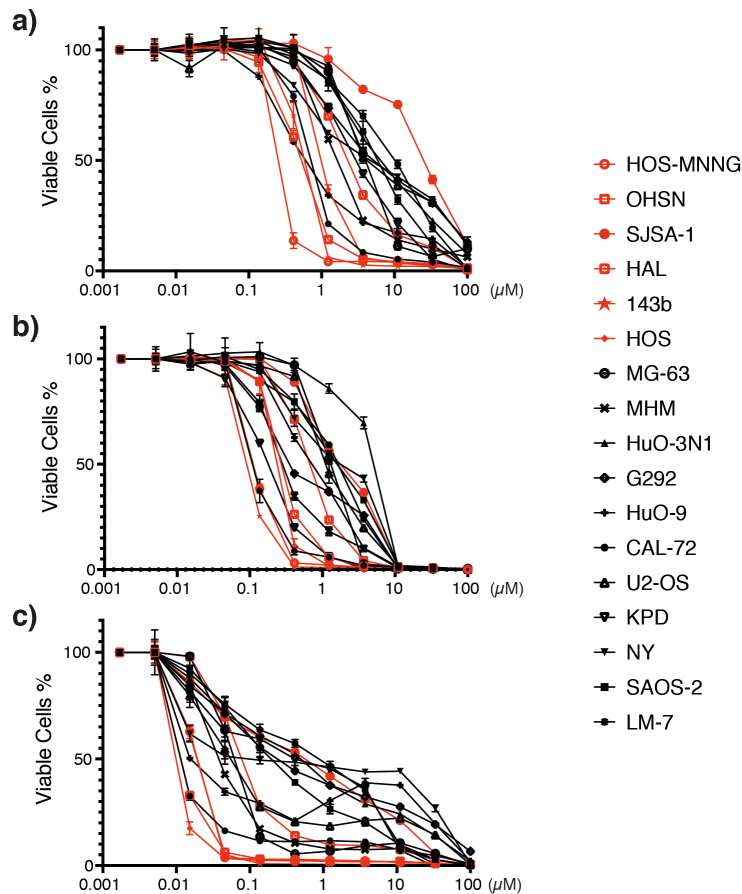
Figure S-3 – ATRi concentration- survival curves in osteosarcoma lines
Graph shows cell viability data used to generate AUC Data in Figure 3 to (a) ATRi AZD-6738, (b) VE-822 and (c) BAY-1895344. Osteosarcoma cell lines are grouped according to telomere length. Individual lines are represented by symbols on the right (short telomere lines n=6, long telomere lines n=11). Short telomere lines are highlighted in red. Curves are from one representative run of two or more runs. Data points represent mean of 3 technical replicates with error bars showing standard deviation.
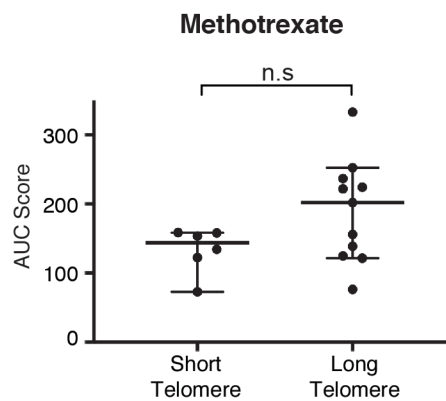

Figure S-4 – Relationship between sensitivity to methotrexate and telomere length
Graph shows AUC values deduced for dose response curves to methotrexate. Lines are grouped according to telomere length cell lines. Samples were assessed in triplicate and values averaged. Bars depict median and 95% CI. Mann-Whitney U Test specified p=0.1802, indicating no significant difference between groups.
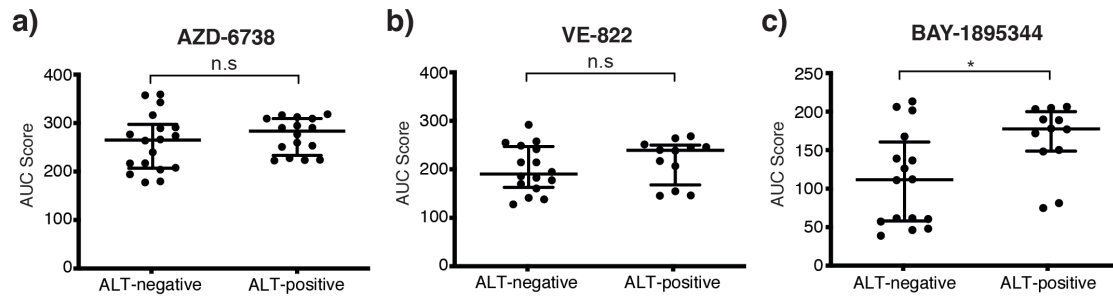
Figure S-5 – Comparison of ATRi sensitivities between ALT negatives and positives
Graph shows sensitivity of osteosarcoma lines to ATRi (a) AZD-6738, (b) VE-822 and (c) BAY-1895344, grouped by ALT status. Data represent AUC deduced from concentration response survival curves. Values summarise outcome from two repeats for each cell line. Bars indicate median AUC and 95% confidence interval for each group. Mann-Whitney U test specified (a) p=0.3008 (ns), (b) p=0.3180 (ns), (c) p=0.0197 (*).
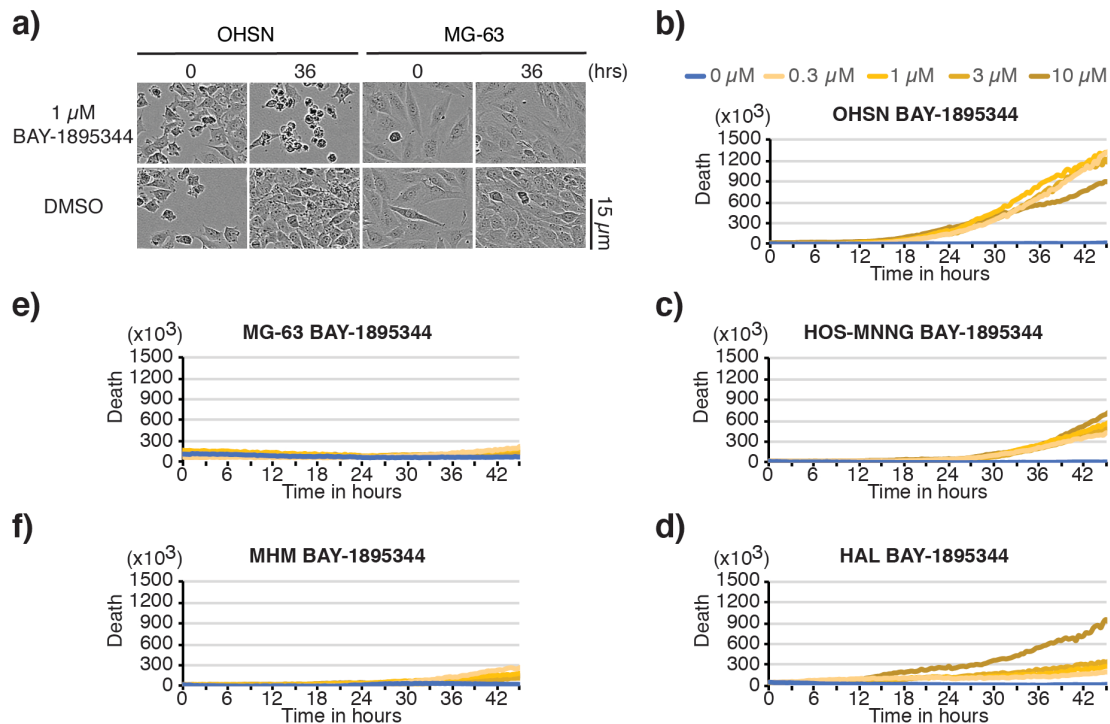
Figure S-6 – Selective death of osteosarcoma with short telomeres exposed to ATR inhibitor BAY-1895344
Short telomere cell lines, OHSN, HOS-MNNG and HAL, and long telomere cell lines, MG-63 and MHM, were treated with VE-822 at the concentration of 0 µM, 0.3 µM, 1 µM and 3 µM and were monitored by *IncuCyte live cell analysis*.
a) Representative images of OHSN and MG-63 with DMSO only and with 1 µM BAY-1895344 at the indicated time are shown. Sample analysis was in the absence of SYTOX™ green death dye and fluorescence imaging. A 15-µm scale bar is shown.
b-f) Representative graphs showing net death over-time for b) OHSN, c) HOS-MNNG, d) HAL, e) MG-63 and f) MHM. Graphs represent one of n=2 independent datasets.

Supplementary References:

1. Fodstad, O., Brogger, A., Bruland, O., Solheim, O. P., Nesland, J. M., and Pihl, A. (1986) Characteristics of a cell line established from a patient with multiple osteosarcoma, appearing 13 years after treatment for bilateral retinoblastoma, *International journal of cancer. Journal international du cancer 38*, 33-40. DOI: 10.1002/ijc.2910380107.

2. Kjonniksen, I., Winderen, M., Bruland, O., and Fodstad, O. (1994) Validity and usefulness of human tumor models established by intratibial cell inoculation in nude rats, *Cancer research 54*, 1715-1719.

3. Bruland, O. S., Fodstad, O., Stenwig, A. E., and Pihl, A. (1988) Expression and characteristics of a novel human osteosarcoma-associated cell surface antigen, *Cancer research 48*, 5302-5309.

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