Table W1. Statistical Analyses for Results Presented in Figure 4.

Cell Line	Time (h)	Estimated *R*	SD of Estimated *R*	*Z*	P	Significance Using Bonferroni Correction
TT	0	1	0	N/A	N/A	
	4.5	0.414	0.012	48.043	.0	*
H9	0	1.000	0.000	N/A	N/A	
	1	0.921	0.055	1.426	.154	
	2	0.691	0.053	5.866	.000	*
	4	0.636	0.018	19.737	.000	*
	8	0.057	0.001	643.575	.000	*
BJ	0	1.000	0.000	N/A	N/A	
	5	0.534	0.020	23.068	.000	*
	9	0.434	0.028	20.375	.000	*
	14	0.213	0.003	244.630	.000	*
RG2	0	1.000	0.000	N/A	N/A	
	5	0.213	0.010	74.463	.000	*
	7	0.114	0.001	831.531	.000	*
	14	0.007	0.001	12735.210	.000	*
MG63	0	1.000	0.000	N/A	N/A	
	3	0.631	0.042	8.693	.000	*

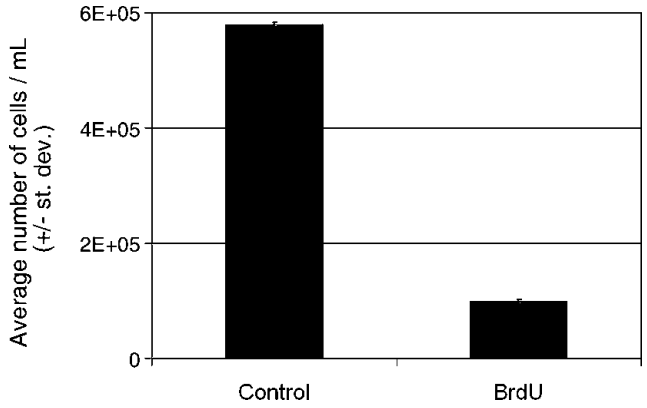

Figure W1. Expansion suppression is not due to BrdU-mediated photolysis. Because BrdU has been shown to increase the sensitivity of cells to irradiation, including light, we exposed MG63 human osteosarcoma cells to 50 μM BrdU for 24 hours and cultured them under light protection for 5 days. Even under these conditions, BrdU-treated cells expanded at a significantly slower rate than controls (unpaired *t* test, Welch-corrected, $P < .0001$). $n = 3$ for each group. Error bars represent SD.

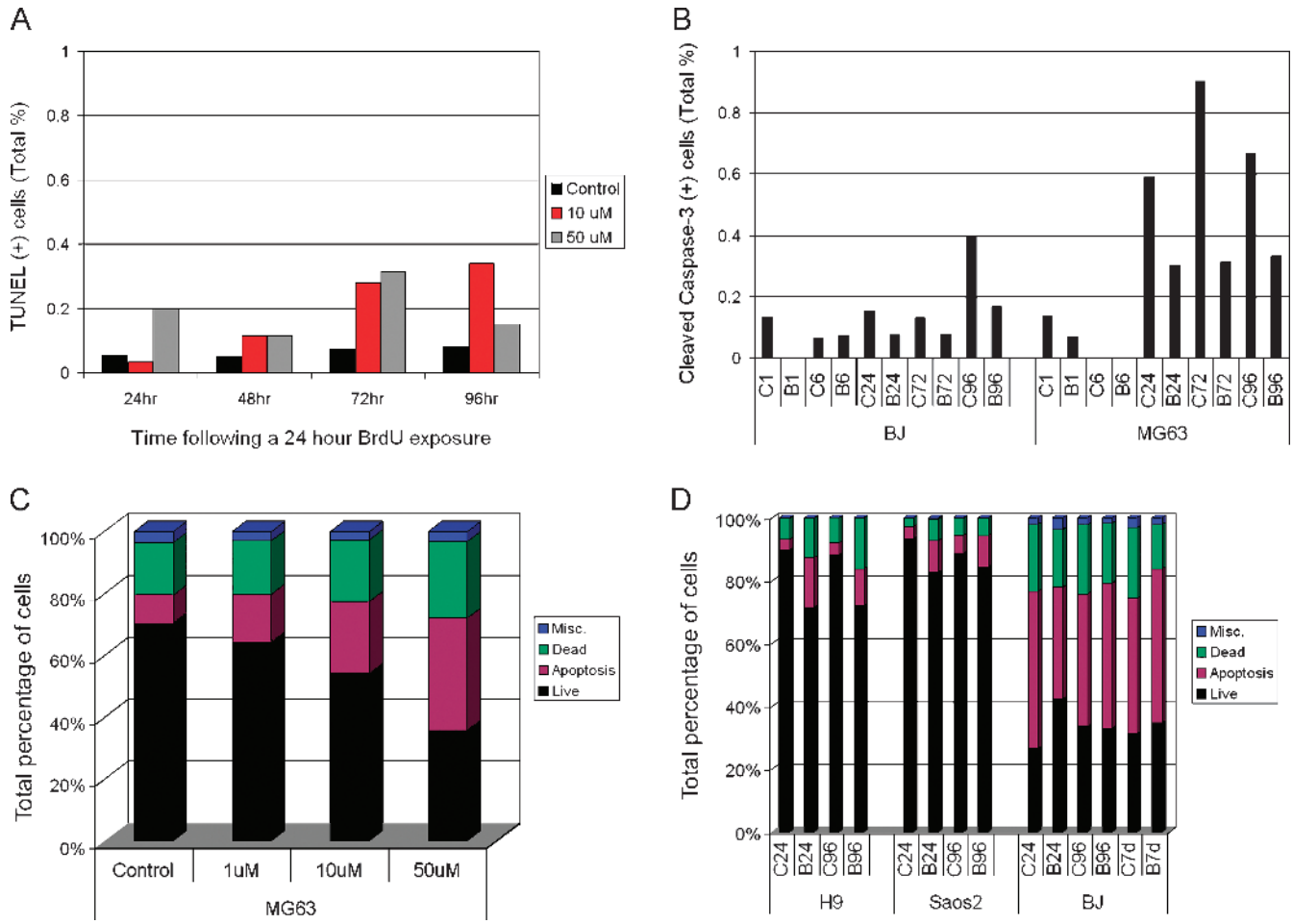

Figure W2. BrdU induces a negligible increase in apoptotic cell death. (A) Apoptosis was assessed with the TUNEL assay in RG2 rat glioma cells that received a 24-hour pulse of either 10 or 50 μM BrdU. At all time points, after BrdU exposure, treated groups show significant increases in TUNEL+ cells compared to control. However, even the highest rate of apoptosis represents less the 0.5% of the total cell number. (B) Cleaved caspase-3 was assessed in control (*C*) and BrdU-treated (*B*) BJ and MG63 cells at 1, 6, 24, 72, and 96 hours after a single 24-hour exposure to 50 μM BrdU. In none of the groups does the percentage of caspase-3+ cells exceed 1% of the total population, and there are no consistent differences between treated and control groups in either cell line. (C) Annexin V labeling (purple) shows a dose-responsive increase in MG63 cells exposed for 24 hours to 1, 10, or 50 μM BrdU. In addition, there is a slight increase in dead cells (green) with increasing concentration of BrdU. (D) Annexin V labeling was assessed in control (*C*) and treated (*B*) H9, Saos-2, and BJ cells at various times after a single 24-hour exposure to 50 μM BrdU. H9, but not Saos-2 or BJ cells show an increase in Annexin V label (purple) by treated cells. Additionally, untreated control cells show wide variability in the constitutive level of Annexin V labeling, with approximately 50% of control BJ cells labeled positive.

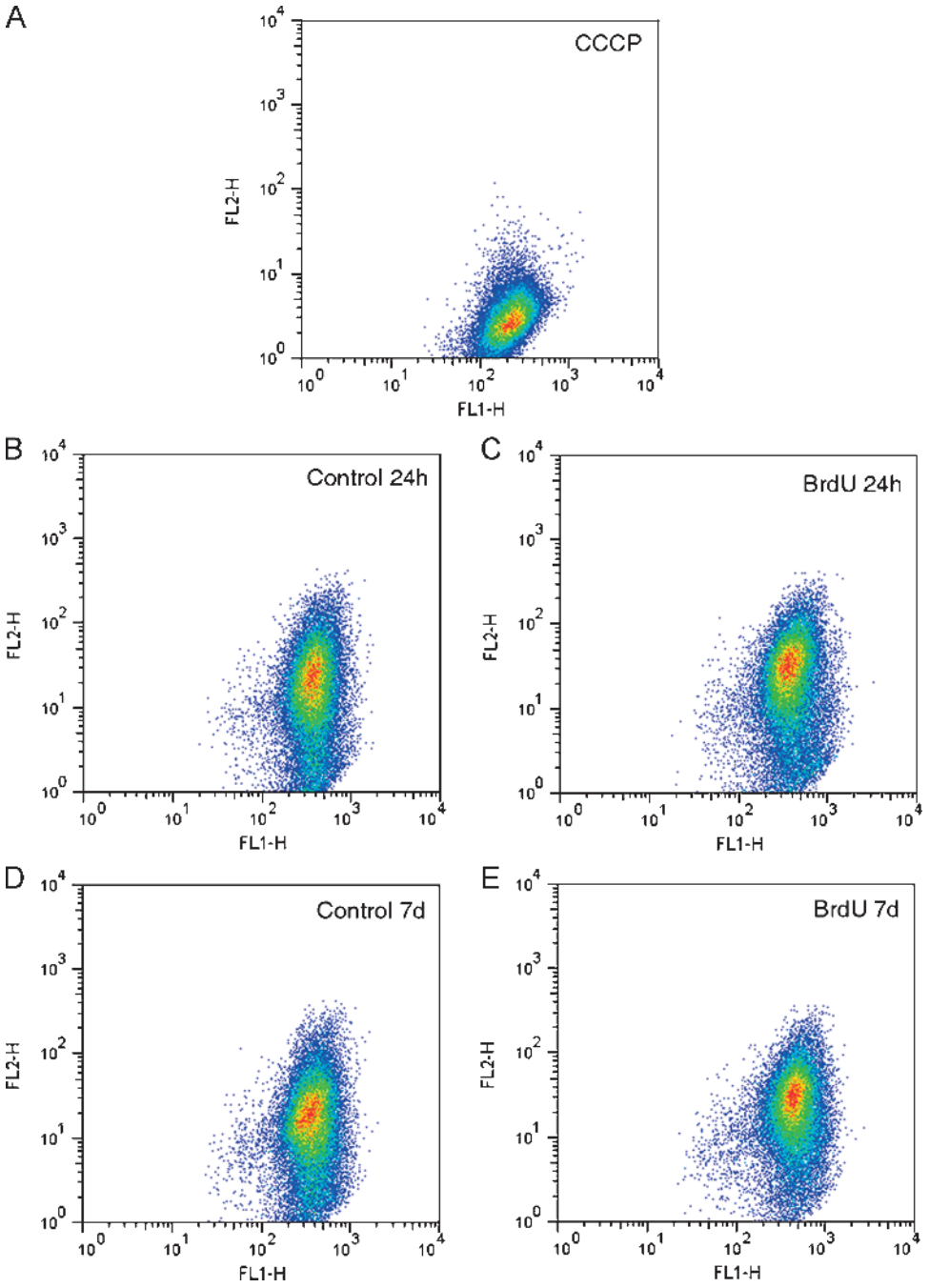

Figure W3. BrdU does not perturb mitochondrial membrane physiology. MG63 human osteosarcoma cells received a 24-hour pulse of 50 μM BrdU, and mitochondrial membrane physiology was assessed using the JC-1 potentiometric dye. Mitochondrial membrane depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. (A) Control MG63 cells were treated with CCCP (a mitochondrial membrane disrupter) as a positive control for depolarization. At both 24 hours (B and C) and 7 days (D and E) after BrdU exposure, control and treated cells display equivalent membrane potentials.

Table W2. Reported Statuses of Prominent Senescence-Related Markers for All Cell Lines Tested.

Cell Line	Description	p53	p16	p21	pRb	Telomerase
H9	Human lymphoma	Mutant	–/–	Not expressed	Normal	Positive
RG2	Rat glioma	–/–	–/–	Normal	N/A	Positive
MG63	Human osteosarcoma	–/–	–/–	Normal	Normal	Positive
BJ	Human immortalized fibroblasts	Normal	Normal	Normal	Normal	Negative
Saos-2	Human osteosarcoma	–/–	Normal	Not expressed	–/–	Negative
TT	Human thyroid cancer	Mutant	N/A	N/A	N/A	Positive

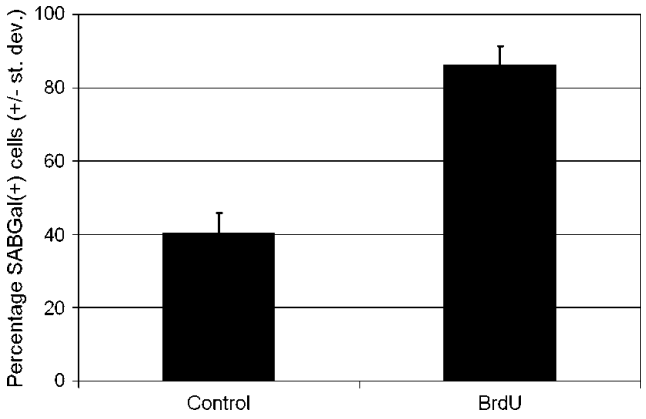

Figure W4. BrdU induces an increase in SAβ-gal activity. RG2 rat glioma cells treated with 50 μM BrdU for 24 hours show an increase in the percentage of SAβ-gal+ cells within 24 hours. Error bars represent SD.

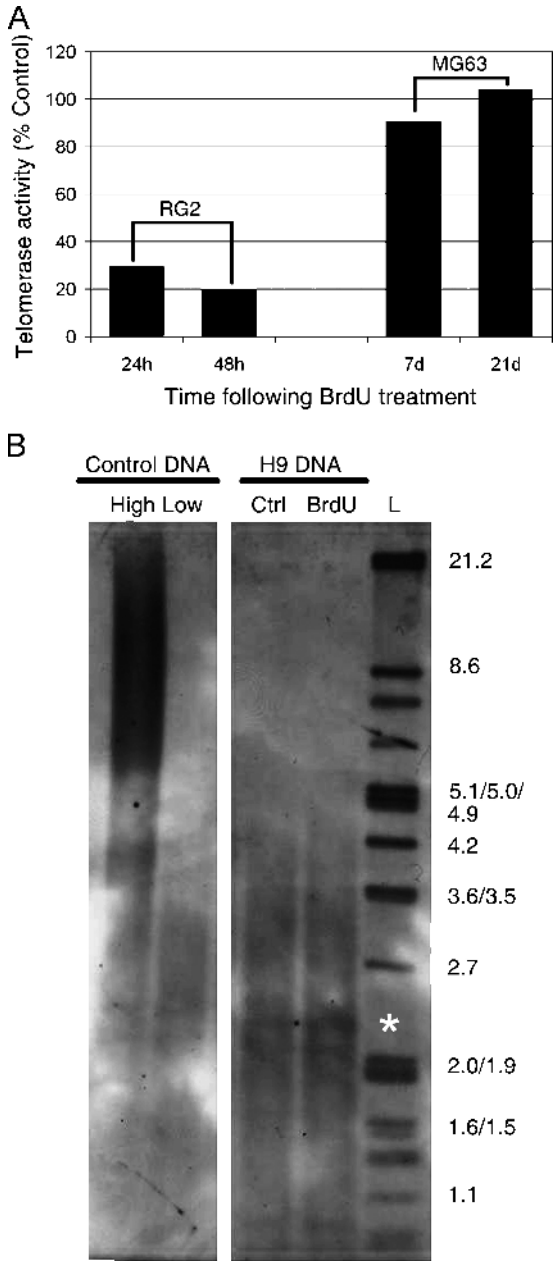

Figure W5. BrdU has varying effects on telomerase expression but does not alter telomere length. (A) RG2 rat glioma and MG63 human osteosarcoma cells were assayed for telomerase expression using TRAP analysis after a 24-hour pulse of 50 μM BrdU. RG2 cells show a dramatic and statistically significant reduction ($P < .001$ at 24 and 48 hours) in telomerase activity within 24 hours of BrdU exposure, whereas telomerase in treated MG63 cells does not vary from control levels even at 7 or 21 days after BrdU when proliferation is severely suppressed. (B) Telomeric terminal restriction fragment was performed on H9 human lymphoma cells 7 days after a 24-hour pulse of 50 μM BrdU. There is no discernible difference in telomere length between the control and BrdU-treated cells.