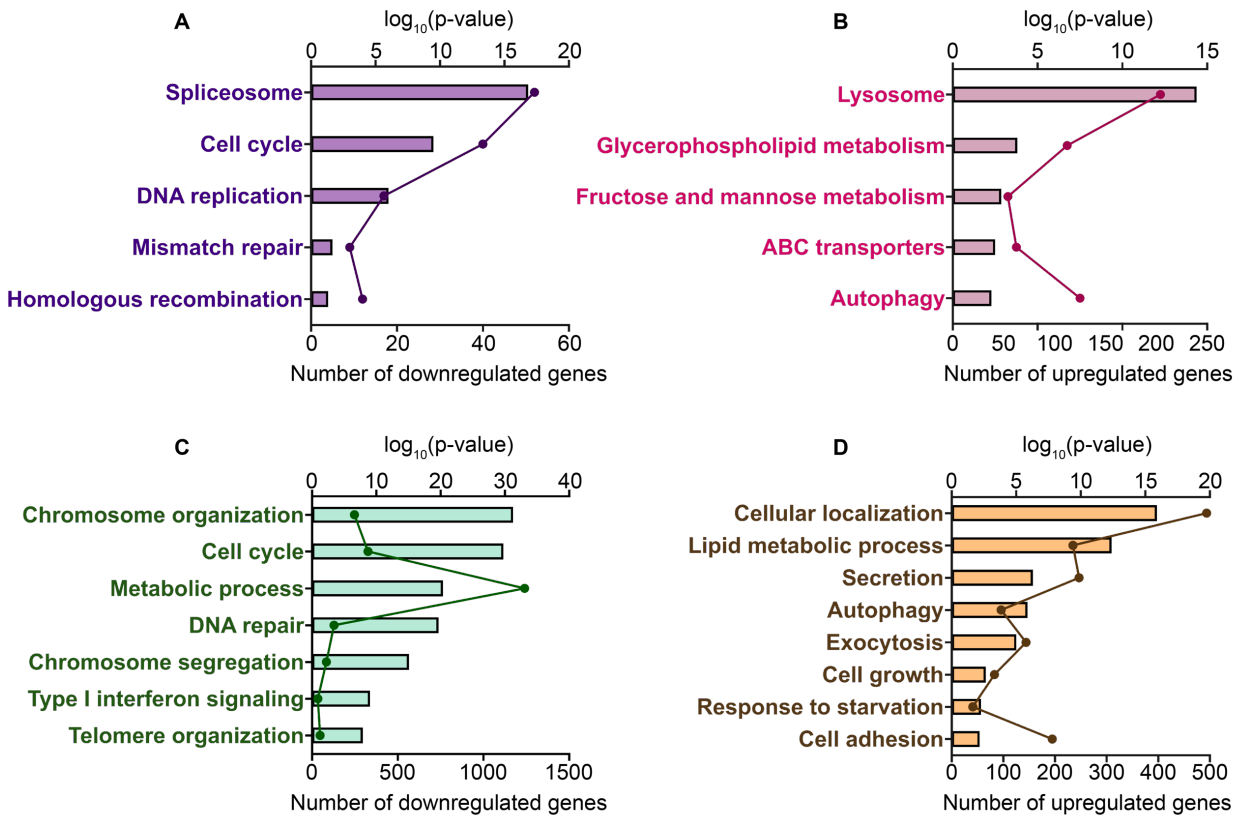

E

Ingenuity Canonical Pathways	-log(p-value)	Up/Down
Interferon Signaling	5.07	↓
NER Pathway	4.46	↓
NRF2-mediated Oxidative Stress Response	4.24	↑
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	3.95	↑
Triacylglycerol Biosynthesis	3.49	↑
Role of BRCA1 in DNA Damage Response	3.45	↓
Pyrimidine Ribonucleotides De Novo Biosynthesis	3.12	↑
Sirtuin Signaling Pathway	3.08	↑

Figure S1 NU-1 treatment alters signaling pathways in MCF7 cells, Related to Figure 1

(A-D) GO term enrichment analysis of differentially expressed genes (DEGs) in NU-1 treated MCF7 cells using DAVID bioinformatics resource confirms enriched pathways identified with Reactome. **A** and **B,** enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in downregulated (**A**) and upregulated (**B**) genes. **C** and **D,** enrichment of Biological Process (BG) GO annotations in downregulated (**C**) and upregulated (**D**) genes. Dots indicate the number of DEGs and bars indicate the -Log_{10}(p-value) for each enriched pathway.

(E) Ingenuity canonical pathway analysis (IPA) of DEGs in NU-1 treated MCF7 cells.

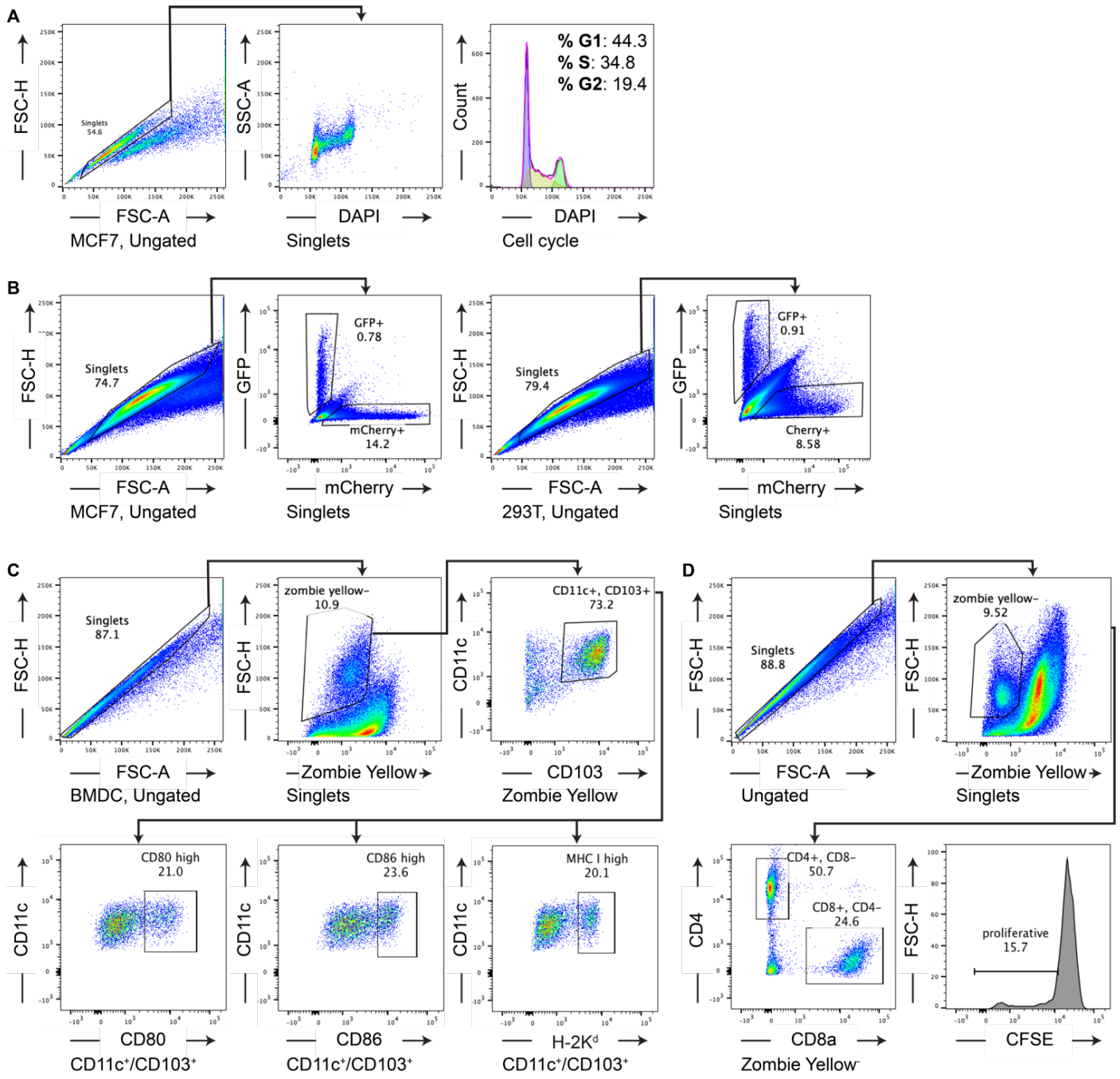

Figure S2 Gating strategy for flow cytometric analysis, Related to STAR Methods

(A) For cell cycle analysis, MCF7 single-cell population was selected based on forward and side scatter (FSC and SSC). The DNA content was analyzed by the intensity of DAPI staining.

(A) In Traffic Light Reporter assays, MCF7 (right) or 293T (left) cells were first gated for singularity and then analyzed for mCherry and GFP expression.

(C) In bone marrow-derived dendric cells (BMDCs) activation assays, BMDCs were first gated for singularity. Then live cells were identified by size and Zombie yellow exclusion and gated on co-expression of CD11c and CD103 to identify DCs. The DC population was gated for expression of activation/maturation markers CD80, CD86, and H-2K^{d}.

(D) In T cell priming assays, cells were first gated for singularity, size, and viability and gated into CD8^{+} and CD4^{+} T cell populations. CFSE dilution was measured in both CD8^{+} and CD4^{+} T cells.

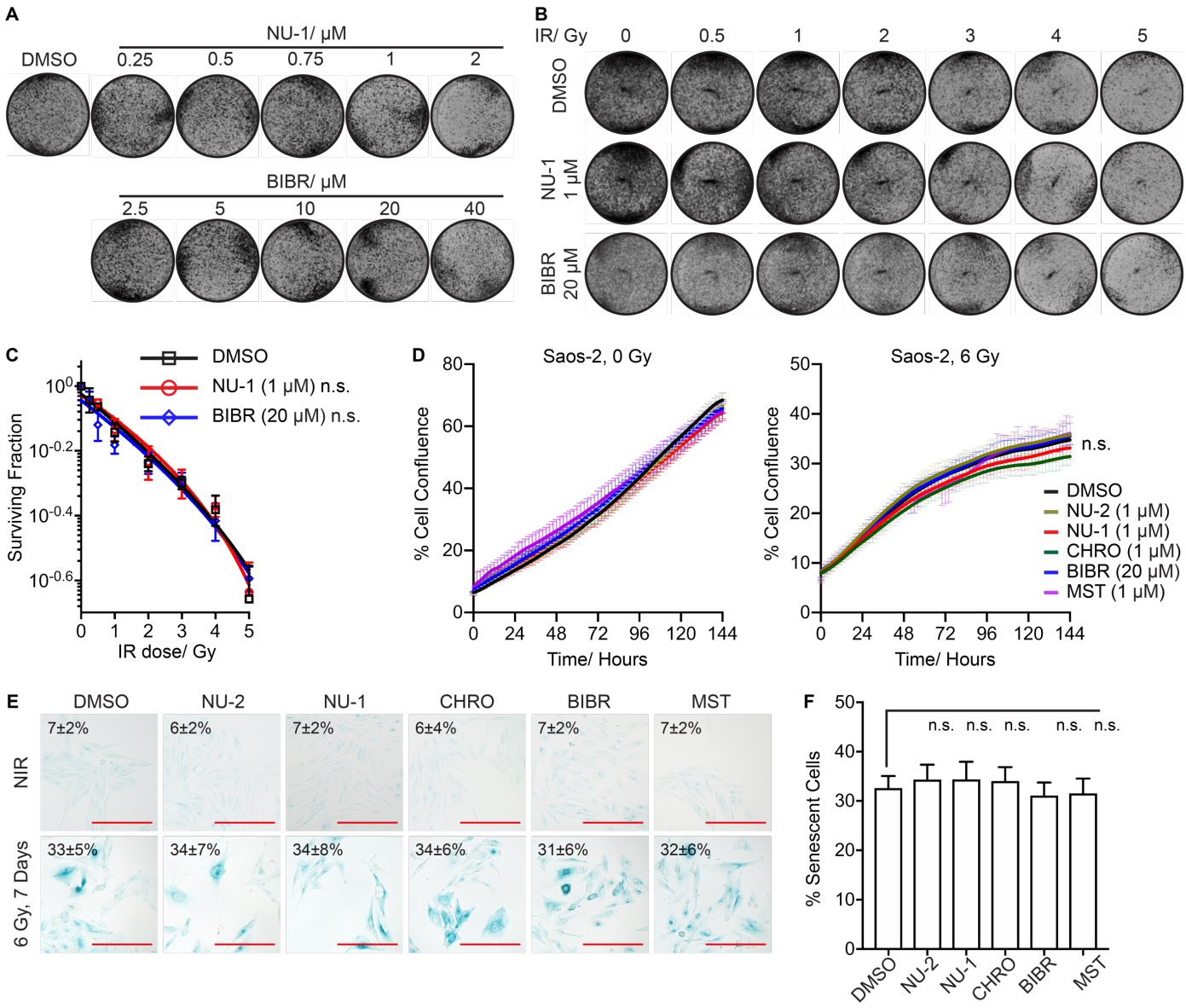

Figure S3 TERT inhibition sensitizes telomerase positive cells to radiation, Related to STAR Methods and Table S3

(A) Clonogenic survival of Saos-2 cells treated with DMSO, NU-1 or BIBR at indicated doses.

(B) Clonogenic survival of Saos-2 cells treated with IR ± TERT inhibitors. Saos-2 cells were treated with DMSO control, NU-1 (1 μM), or BIBR (20 μM) for 1 h, then irradiated at the indicated doses. Shown are representative images from three replicates.

(C) Quantitative analysis of **B**. Data from three replicates, mean ± SD.

(D) Automated proliferation analysis from time-lapse imaging over 6 days comparing Saos-2 cells treated with DMSO, NU-2, NU-1, CHRO, BIBR or MST for 1 h, followed by 0 (NIR, left) or 6 Gy (right) at time 0. Results are shown as mean ± SD. Images of 25 non-overlapping fields were captured for analysis of each sample.

(E) SA-β-Gal staining of Saos-2 cells. Saos-2 cells were treated as in **D**, then fixed and stained after 7 days. Shown are representative images. % of SA-β-Gal$^+$ cells are indicated. Scale bars=200 μm.

(F) Quantification of SA-β-Gal-positive Saos-2 cells after IR. Data from 5 non-overlapping images, mean ± SD. n.s. $P > 0.05$ compared to DMSO (unpaired t-test).

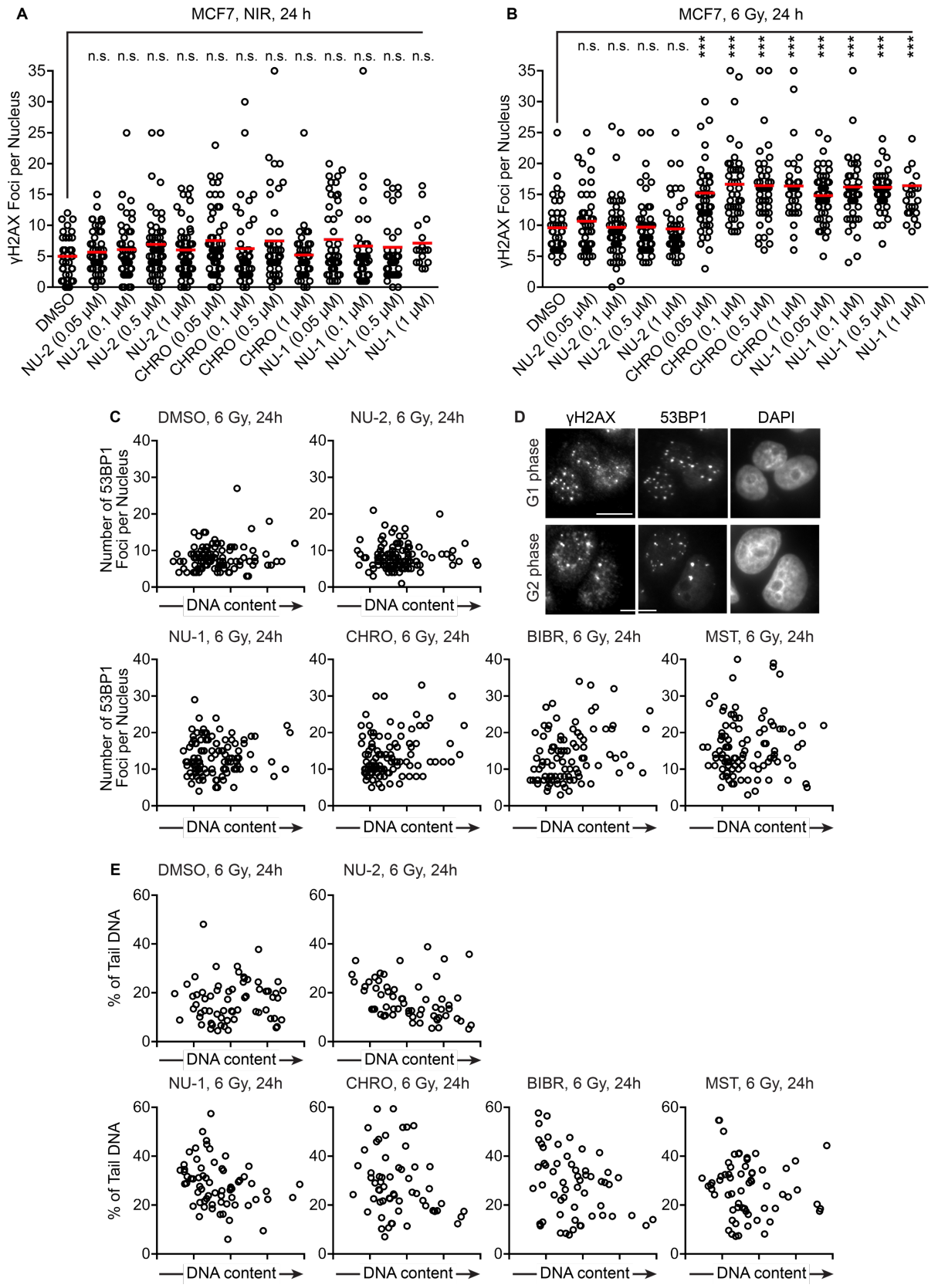

Figure S4 TERT inhibition promotes DNA damage foci persistence in MCF7 cells, Related to Figure 4

(A and B) Quantification of γH2AX foci in MCF7 cells. Cells were treated with DMSO, CHRO, NU-1, or NU-2 at indicated doses for 1 h, followed by 0 (NIR, **A**) or 6 Gy (**B**), fixed after 24 h and stained for γH2AX. >30 cells were analyzed for each condition. Shown are individual cells (open

circles) and mean (red bar). *** $P < 0.001$; n.s. $P > 0.05$ compared to DMSO (unpaired t-test).

(C) DNA damage foci staining assays with MCF7 cells were performed as in **Figure 4A** and **B**. The number of γH2AX foci and DNA content based on DAPI intensity are plotted for individual cells (open circles).

(D) Representative images of MCF7 cells with G1 and G2 DNA content. MCF7 cells were treated with NU-1 (0.5 μM) for 1 h, followed 6 Gy irradiation, then fixed and stained after 24 h. Scale bars, 20 μm.

(E) Neutral comet assays with MCF7 cells were performed as in **Figure 4H**. The % tail DNA and total DNA content are plotted for individual nuclei (open circles).

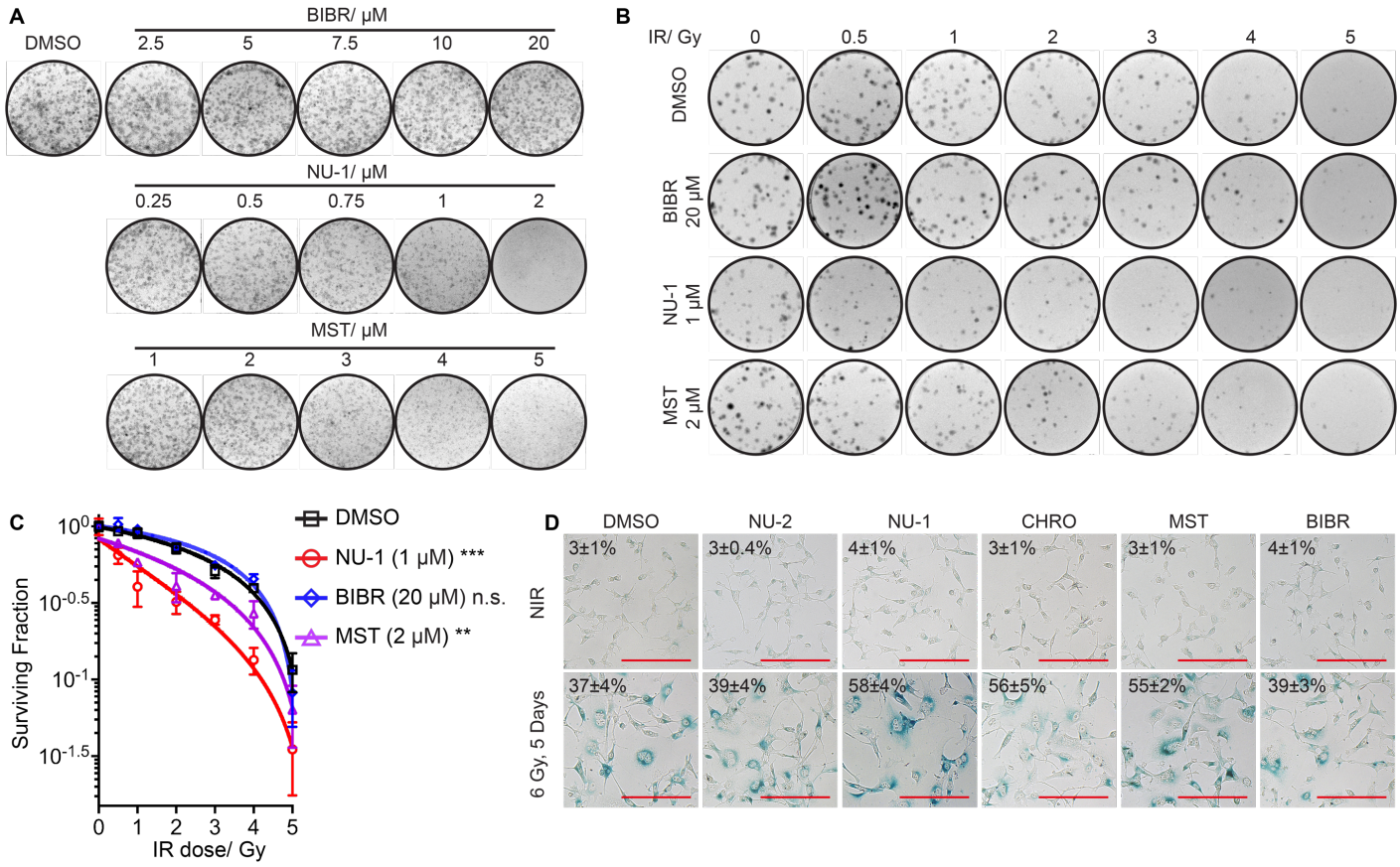

Figure S5 TERT inhibition sensitizes telomerase positive cells to radiation, Related to STAR Methods and Table S3

(A) Clonogenic assay of CT26 cells after telomerase inhibition. CT26 cells were treated with DMSO control, NU-1, BIBR, or MST at the indicated concentrations. Performed in triplicate. Representative images are shown.

(B) Clonogenic survival of CT26 cells after irradiation in the presence or absence of inhibitors. CT26 cells were treated with DMSO, BIBR (20 μM), NU-1 (1 μM), or MST (2 μM) for 1 h, followed by irradiation at the indicated doses. Performed in triplicate. Representative images are shown.

(C) Quantitative analysis of **B**. Normalized surviving fractions indicating the average of three replicates are shown, mean ± SD. *** $P < 0.001$; ** $0.001 < P < 0.01$; n.s. $P > 0.05$ compared to DMSO (unpaired t-test).

(D) SA-β-Gal staining of CT26 cells. CT26 cells were treated with DMSO, NU-2, NU-1, CHRO, BIBR or MST for 1 h, followed by irradiation at 0 (NIR) or 10 Gy. Cells were fixed and stained 5 days after radiation. Representative 20X images are shown. Percentage of SA-β-Gal$^+$ cells are indicated. Scale bars=200 μm.

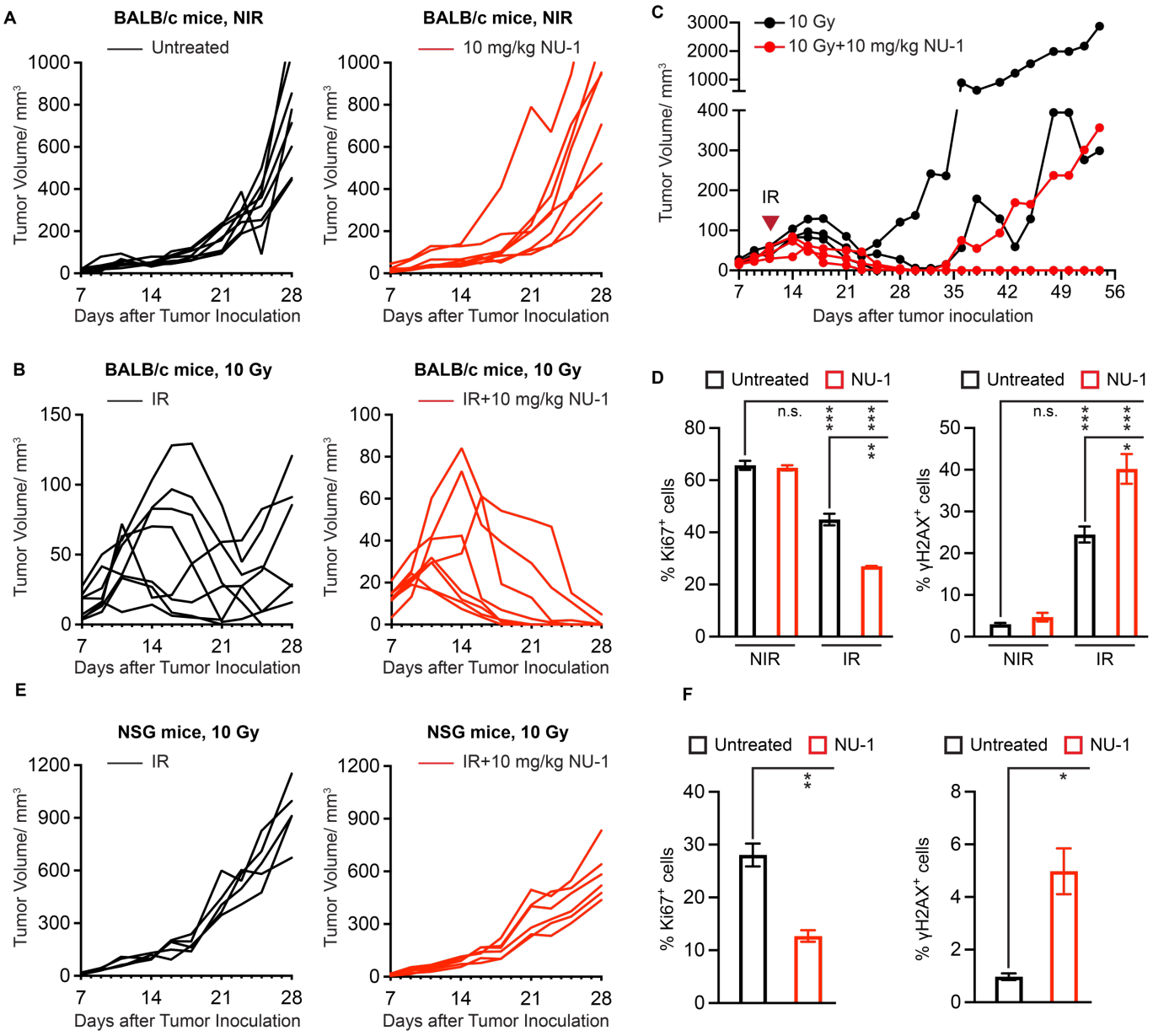

Figure S6 TERT inhibition sensitizes CT26 tumors to radiation, Related to Figure 6

(A-C) Growth curve of individual CT26 tumors in BALB/c mice. **A** and **B**, Tumor growth in individual BALB/c mice shown in **Figure 6B** and **6C**. **C**, Tumors were measured for 54 days after CT26 cell inoculation in BALB/c mice after radiation alone or combined with NU-1 (n = 3 for each).

(D) Bar graphs showing quantification of proliferating cells (Ki67$^+$) and DNA damage (γH2AX$^+$) in stained tumors shown in **Figure 6E.** Data from >3 non-overlapping images, mean ± SEM.

(E) Tumor growth in individual NSG mice as shown in **Figure 6H.**

(F) Bar graphs showing quantification of proliferating cells (Ki67$^+$) and DNA damage (γH2AX$^+$) in tumors stained as in **Figure 6J.** Data from >3 non-overlapping images, mean ± SEM. *** P < 0.001; ** 0.001 < P < 0.01; * 0.01 < P < 0.05; n.s. P > 0.05 compared to DMSO (unpaired t-test).

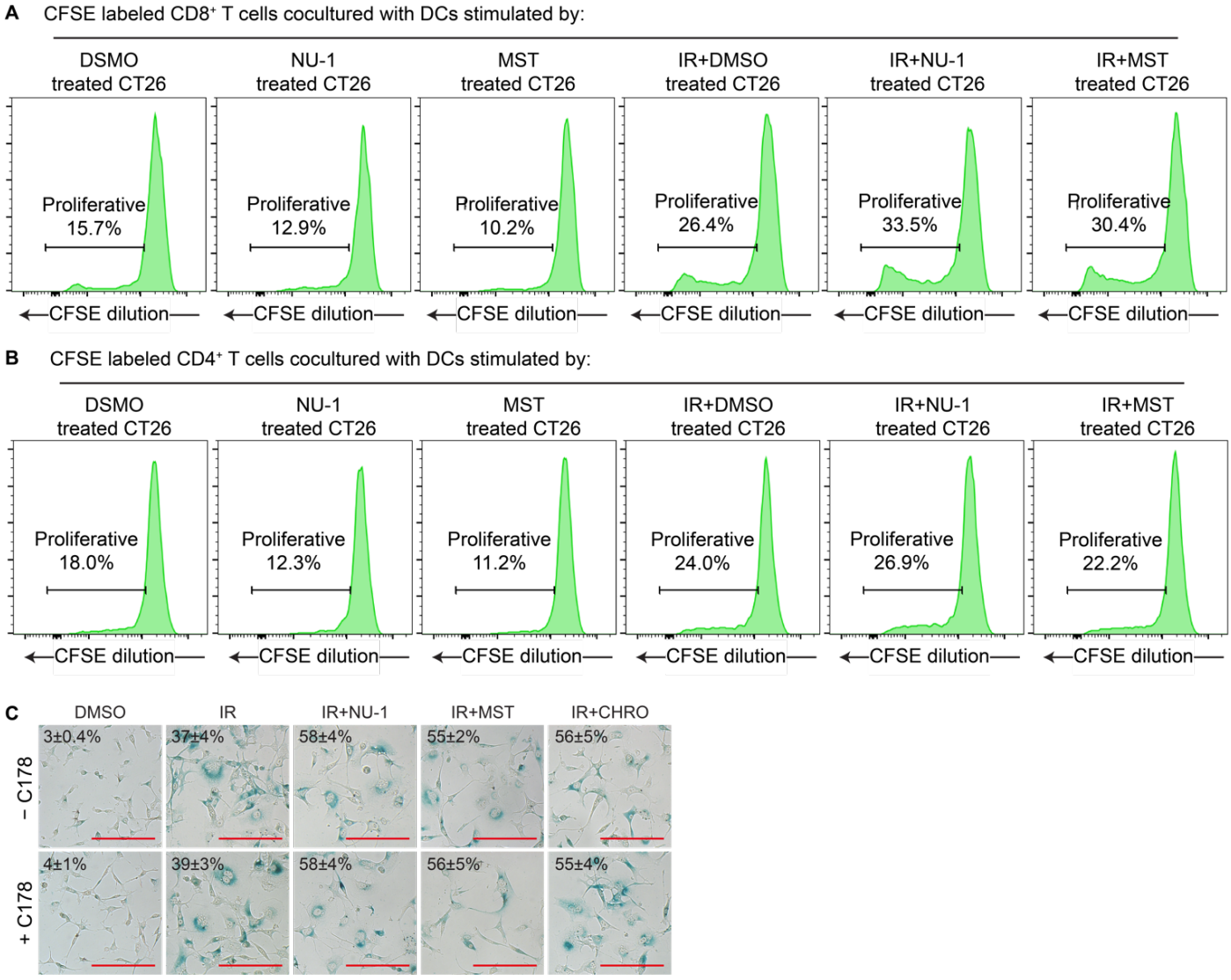

Figure S7 Coculture with IR+TERT inhibitors treated CT26 cells enables dendritic cells to prime T cells, Related to Figure 7

(A and B) Assays of DCs stimulated by CT26 cells for activation of $CD8^+$ (**C**) or $CD4^+$ (**D**) T cell proliferation. As indicated in **Figure 7C** and **D**, CFSE-labeled murine splenocytes were cocultured for 5 days with DCs pre-stimulated by CT26 cells treated with DMSO, NU-1, MST alone or combined with 10 Gy. Shown are histograms of Zombie $yellow^-/CD8^+/CD4^-$ T cell population (**C**) and Zombie $yellow^-/CD8^-/CD4^+$ T cell population (**D**).

(C) SA-β-Gal staining of CT26 cells. CT26 cells were treated with IR (10 Gy), IR + NU-1 (1 μM), IR + MST (2 μM), IR + CHRO (1 μM), or DMSO control in the presence or absence of STING inhibitor C178 (4 μM). Cells were fixed and stained 5 days after irradiation. Representative 20X images are shown. Percentage of SA-β-Gal^+ cells are indicated (mean ± SD). Scale bars=200 μm.

Table S1 Viability of cells treated with chemotherapy ± TERT inhibitors, Related to Figure 2

	Viability	SD	Viability	SD	Viability	SD	Viability	SD	Viability	SD
MCF7 cells										
log[Irinotecan], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**		**+ BIBR 1532**		**+ MST-312**	
-3.000	94.667	3.055	94.667	4.509	86.667	1.528	91.000	1.000	90.333	2.082
-2.000	84.333	2.517	85.000	4.583	78.333	1.528	83.667	2.887	82.667	1.528
-1.000	62.000	2.000	59.333	6.506	41.333	1.528	53.333	3.215	53.333	2.082
0.000	37.000	2.000	36.000	6.000	15.000	3.606	27.333	5.033	27.333	2.517
1.000	10.000	2.000	10.667	5.033	6.667	1.528	12.3333	5.132	10.000	2.000
2.000	2.000	1.000	2.000	2.000	1.333	0.577	2.000	1.000	2.000	1.000
log[Etoposide], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
-3.000	94.667	3.055	97.000	2.000	94.000	2.000				
-2.000	85.000	3.000	85.333	4.509	86.000	2.000				
-1.000	75.333	4.509	71.333	6.110	36.000	4.583				
0.000	27.333	4.726	32.000	6.083	7.667	3.512				
1.000	10.000	2.000	10.667	5.033	6.667	1.528				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
log[Doxorubicin], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	94.667	3.055	94.333	4.509	94.000	2.000				
0.500	93.000	2.646	92.667	5.033	86.667	5.508				
1.000	68.667	4.509	63.667	5.508	44.333	3.055				
1.500	9.333	2.887	8.333	3.786	2.000	1.000				
2.000	5.000	3.606	2.667	1.528	2.000	1.000				
2.500	2.000	1.000	2.000	2.000	1.333	0.577				
3.000	1.667	0.577	2.333	1.528	1.333	0.577				
log[Paclitaxel], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	94.667	3.055	94.333	4.509	94.000	2.000				
0.400	92.000	1.000	88.333	3.055	89.333	4.041				
0.800	80.000	4.000	78.000	3.000	51.667	4.509				
1.200	15.667	5.033	22.333	7.638	5.000	2.000				
1.600	2.333	1.528	4.333	4.163	2.000	1.000				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
2.500	1.667	0.577	2.333	1.528	1.333	0.577				
3.000	0.000	0.000	0.000	0.000	0.000	0.000				
Co-administration										
log[Irinotecan], μM	**+ DMSO**		**+ NU-1**		**+ BIBR 1532**		**+ MST-312**			
-3.000	94.667	3.055	98.000	2.000	97.000	1.000	98.667	1.528		
-2.000	93.000	1.000	93.667	1.155	94.333	1.528	93.667	2.082		
-1.000	84.667	2.517	77.667	2.517	78.333	0.577	78.000	3.606		
0.000	29.000	4.000	24.333	2.309	27.333	3.786	26.333	3.215		
1.000	5.000	3.606	8.667	1.528	8.333	0.577	4.000	1.732		
2.000	2.000	1.000	1.000	1.000	2.000	0.000	1.667	0.577		
Post-administration										
log[Irinotecan], μM	**+ DMSO**		**+ NU-1**		**+ BIBR 1532**		**+ MST-312**			
-3.000	94.667	3.055	97.667	2.082	97.333	1.155	97.667	1.155		
-2.000	84.333	2.517	92.333	0.577	95.333	1.155	93.667	0.577		
-1.000	77.000	4.583	71.667	2.082	72.000	2.000	71.000	1.732		
0.000	23.333	5.132	20.333	2.517	23.333	1.155	20.667	2.082		
1.000	7.000	3.606	6.667	0.577	6.667	3.512	9.000	1.000		
2.000	2.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000		
Saos-2 cells										
log[Irinotecan], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**		**+ BIBR 1532**		**+ MST-312**	
-3.000	93.667	1.528	93.000	2.646	92.333	2.082	92.667	2.082	90.333	2.082
-2.000	84.333	2.517	82.000	6.245	83.000	2.000	85.333	2.887	82.667	1.528
-1.000	63.000	3.606	65.333	5.033	59.000	2.000	61.000	8.660	65.333	4.041
0.000	36.667	4.509	35.667	7.638	34.333	4.163	36.333	2.082	37.000	2.000
1.000	8.667	2.082	10.333	5.508	9.333	3.215	10.667	4.041	12.667	2.082
2.000	2.000	1.000	2.000	1.000	3.333	1.155	2.000	1.000	2.000	1.000

log[Etoposide], μM	+ DMSO		+ NU-2		+ NU-1					
-3.000	94.667	3.055	97.000	2.000	94.000	2.000				
-2.000	87.667	4.933	91.000	3.606	87.667	4.933				
-1.000	81.000	6.557	76.333	5.508	75.000	5.568				
0.000	12.667	3.055	16.667	6.110	15.667	5.033				
1.000	5.000	2.646	4.333	1.528	2.333	1.528				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
log[Doxorubicin], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	102.000	2.000	96.333	2.517	100.333	1.528				
0.500	93.667	3.786	92.667	5.033	92.000	2.000				
1.000	50.333	3.512	51.000	7.550	46.333	7.095				
1.500	2.333	1.528	0.000	0.000	0.000	0.000				
2.000	2.000	1.000	0.000	0.000	0.000	0.000				
2.500	0.000	0.000	0.000	0.000	0.000	0.000				
3.000	0.000	0.000	0.000	0.000	0.000	0.000				
log[Paclitaxel], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	94.667	3.055	94.333	4.509	93.000	2.000				
0.400	90.333	2.517	87.667	6.506	92.000	2.000				
0.800	68.333	3.055	61.333	3.215	55.667	3.215				
1.200	12.333	2.517	10.333	6.110	6.000	4.583				
1.600	2.333	1.528	4.333	4.163	2.000	1.000				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
2.500	1.667	0.577	2.333	1.528	1.333	0.577				
3.000	0.000	0.000	0.000	0.000	0.000	0.000				
A549 cells										
log[Irinotecan], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
-3.000	96.000	2.000	94.333	2.517	94.000	2.000				
-2.000	88.000	3.000	88.667	3.215	88.333	3.055				
-1.000	79.667	5.033	72.000	2.646	46.000	3.606				
0.000	35.333	4.509	31.667	8.622	15.333	3.512				
1.000	13.667	3.215	8.000	4.583	4.667	3.055				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
log[Etoposide], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
-3.000	94.667	3.055	97.000	2.000	94.000	2.000				
-2.000	84.000	4.583	90.333	2.082	86.333	5.686				
-1.000	83.667	1.528	81.667	6.506	52.333	5.508				
0.000	15.333	6.110	15.667	8.622	5.000	3.606				
1.000	3.000	1.732	3.667	2.517	2.000	1.000				
2.000	2.000	1.000	2.000	2.000	1.333	0.577				
log[Doxorubicin], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	100.667	2.082	97.667	3.215	99.333	3.512				
0.500	97.667	4.509	96.667	4.933	88.667	4.163				
1.000	79.333	3.512	72.000	3.000	50.667	5.033				
1.500	16.667	5.508	16.000	7.000	0.000	0.000				
2.000	1.000	1.732	2.667	1.528	0.000	0.000				
2.500	0.000	0.000	2.000	2.000	0.000	0.000				
3.000	0.000	0.000	2.333	1.528	0.000	0.000				
log[Paclitaxel], μM	**+ DMSO**		**+ NU-2**		**+ NU-1**					
0.000	94.667	3.055	102.333	2.082	102.333	3.055				
0.400	88.000	7.000	99.667	2.082	92.000	4.000				
0.800	87.333	3.512	81.333	2.517	87.333	4.041				
1.200	43.000	5.292	37.000	7.000	21.667	3.055				
1.600	7.333	4.041	10.667	6.506	2.000	1.000				
2.000	1.000	0.000	0.000	0.000	0.000	0.000				
2.500	1.333	0.577	0.000	0.000	0.000	0.000				
3.000	0.333	0.577	0.000	0.000	0.000	0.000				

Table S2 Lethal dose 50 (LD_{50}) in cells, Related to Figure 2

	MCF7			Saos-2			A549		
	LD_{50}	SEM	P value	LD_{50}	SEM	P value	LD_{50}	SEM	P value
Irinotecan (μM)									
+ DMSO	0.366	0.063		0.399	0.075		0.552	0.089	
+ NU-2	0.292	0.100	0.565	0.443	0.129	0.783	0.414	0.068	0.285
+ NU-1	0.080	0.009	0.011	0.281	0.053	0.268	0.090	0.011	0.007
+ BIBR 1532	0.158	0.038	0.048	0.366	0.099	0.804	N/A		
+ MST-312	0.168	0.025	0.043	0.537	0.071	0.252	N/A		
Etoposide (μM)									
+ DMSO	0.393	0.059		0.306	0.043		0.398	0.065	
+ NU-2	0.389	0.085	0.971	0.279	0.032	0.641	0.323	0.046	0.400
+ NU-1	0.061	0.006	0.005	0.296	0.035	0.866	0.119	0.011	0.013
Doxorubicin (μM)									
+ DMSO	13.650	0.512		9.905	0.263		16.900	0.637	
+ NU-2	12.710	0.538	0.274	10.400	0.270	0.259	15.060	0.697	0.123
+ NU-1	9.525	0.291	0.002	9.529	0.303	0.402	10.310	0.372	0.001
Paclitaxel (μM)									
+ DMSO	10.070	0.831		8.542	0.569		15.140	1.713	
+ NU-2	10.760	1.431	0.698	7.728	0.959	0.506	12.010	1.563	0.248
+ NU-1	6.661	0.382	0.020	6.985	0.972	0.239	11.220	1.254	0.139
Irinotecan (μM) (co-administered with TERT inhibitor)									
+ DMSO	0.426	0.037							
+ NU-1	0.332	0.027	0.122						
+ BIBR 1532	0.371	0.024	0.447						
+ MST-312	0.368	0.027	0.409						
Irinotecan (μM) (post-administered with TERT inhibitor)									
+ DMSO	0.303	0.055							
+ NU-1	0.257	0.017	0.629						
+ BIBR 1532	0.276	0.018	0.878						
+ MST-312	0.248	0.020	0.508						

Table S3 Surviving fraction at 2 Gy (SF_2), Related to Figure 3, S3, and S6

MCF7 cells			
Treatment	Mean SF_2	SD	P value (compared to DMSO treatment)
DMSO	0.369	0.038	N/A
NU-1	0.116	0.045	0.0018
BIBR 1532	0.256	0.030	0.0153
MST-312	0.245	0.035	0.0138
Saos-2 cells			
Treatment	Mean SF_2	SD	P value (compared to DMSO treatment)
DMSO	0.576	0.024	N/A
NU-1	0.583	0.057	0.4419
BIBR 1532	0.580	0.036	0.4495
CT26 cells			
Treatment	Mean SF_2	SD	P value (compared to DMSO treatment)
DMSO	0.721	0.045	N/A
NU-1	0.322	0.044	0.0004
BIBR 1532	0.727	0.020	0.4392
MST-312	0.407	0.071	0.0032