Supplemental Materials

Table S1. Frequency of ts p53 mutations in cancer.

Ts mutation	Cases in COSMIC	% of [a]24,679		Ts mutation	Cases in COSMIC	% of [a]24,679
S99FP	4	0.02%		S215GIN	91	0.37%
Y126C	27	0.11%		V216L	18	0.07%
N131HS	8	0.03%		V217G	6	0.02%
C135W	36	0.15%		P219LS	9	0.04%
A137V	1	0.00%		P223AHLRT	14	0.06%
A138VT	39	0.16%		C229G	1	0.00%
V143A	19	0.08%		T231ANPS	2	0.01%
W146G	3	0.01%		I232FM	13	0.05%
P151AS	136	0.55%		Y234CH	134	0.54%
P152LT	109	0.44%		S240R	7	0.03%
G154V	65	0.26%		M246V	67	0.27%
T155IN	54	0.22%		N247IY	13	0.05%
R156GP	37	0.15%		R249K	20	0.08%
R158CGHL	283	1.15%		P250L	70	0.28%
M160IKRT	9	0.04%		I251LT	4	0.02%
A161GSV	23	0.09%		L252FH	11	0.04%
Q165LPR	5	0.02%		T253AINS	11	0.04%
V172FAGL	46	0.19%		I254FMT	15	0.06%
R175GL	61	0.25%		I255FM	38	0.15%
A189PS	3	0.01%		T256AIR	4	0.02%
P190T	11	0.04%		L257V	9	0.04%
H193RQY	231	0.94%		N268IT	2	0.01%
R196L	3	0.01%		F270IV	27	0.11%
V197LM	16	0.06%		E271AGV	12	0.05%
Y205NH	18	0.07%		V272MAL	203	0.82%
D208AV	22	0.09%		V274A	38	0.15%
T211AP	7	0.03%		R282W	820	3.32%
F212V	2	0.01%		R283HP	46	0.19%
R213GP	14	0.06%		E285KDGV	244	0.99%
H214RDLNY	118	0.48%		E286GKQV	206	0.83%
S215GIN	91	0.37%		L289P	2	0.01%
Total	1501	6.08%		Total	2157	8.74%

a-Total number of tumors with missense p53 mutation in COSMIC.

Table S2. Ts p53 mutation frequency in the COSMIC database.

	All tumors	Lung	Skin	Brain	Ovary	Breast
Missense mutations	24679	2715	709	1295	1278	2191
Ts mutations	3658	500	134	208	152	268
% ts	14.8%	18.4%	18.9%	16.1%	11.9%	12.2%

Table S3. Seventeen most frequent p53 ts mutants.

Validated ts mutants	**Cases in COSMIC**	**[a]Frequency**	**[b]Tumor cell lines**
R282W	820	3.32%	32
E285K	252	1.02%	13
V272M	146	0.59%	10
Y234C	144	0.58%	9
R158H	129	0.52%	9
R158L	127	0.51%	8
P152L	124	0.50%	4
H214R	122	0.49%	3
H193Y	76	0.31%	2
P250L	76	0.31%	1
V272L	69	0.28%	3
G154V	68	0.28%	3
S215I	45	0.18%	4
V274A	41	0.17%	0
A138V	40	0.16%	1
I255F	40	0.16%	1
V172F	39	0.16%	1
Total	**2358**	**9.55%**	

a-Frequency in 24,679 tumors with mutant p53 missense mutations.
b-Mutant tumor cell lines in the IARC TP53 Database.

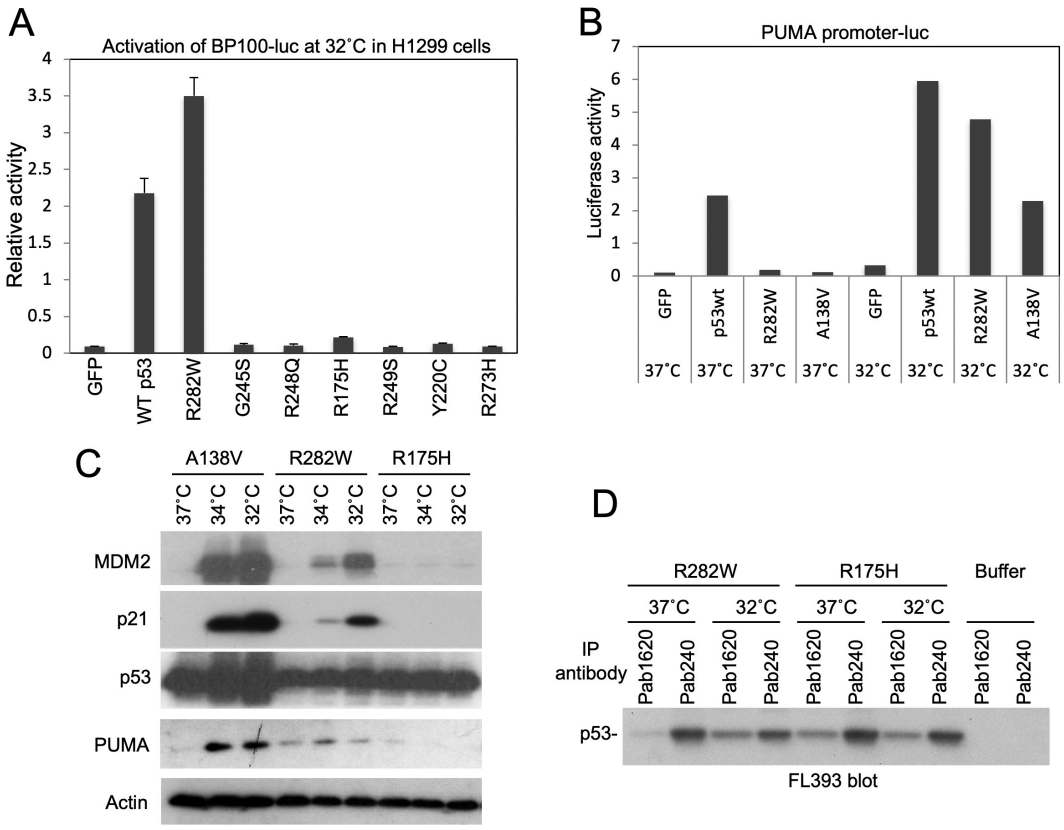

Figure S1. R282W is temperature-sensitive in human cells. (A) H1299 cells were transfected with p53-responsive BP100-luc reporter and p53 hotspot mutants. The cells were cultured at 32˚C for 24 hrs and activation of the reporter was determined. The results were average of 3 experiments. (B) H1299 cells were transfected with PUMA promoter-luc reporter and p53 mutants. The cells were cultured at 37˚C or 32˚C for 24 hrs and activation of the reporter was determined. (C) H1299 cells were stably transfected with indicated plasmids at 37˚C. Cells were cultured at 34˚C or 32˚C for 20 hrs and p53 target gene expression was analyzed by Western blot. (D) H1299 cells expressing p53 mutants were kept at 37˚C or shifted to 32˚C for 18 hrs. P53 from identical amount of extract was immunoprecipitated with wt conformation-specific Pab1620 or mutant conformation-specific Pab240 antibodies and detected by Western blot using pan-specific antibody FL393. R175H was used as non-ts control.

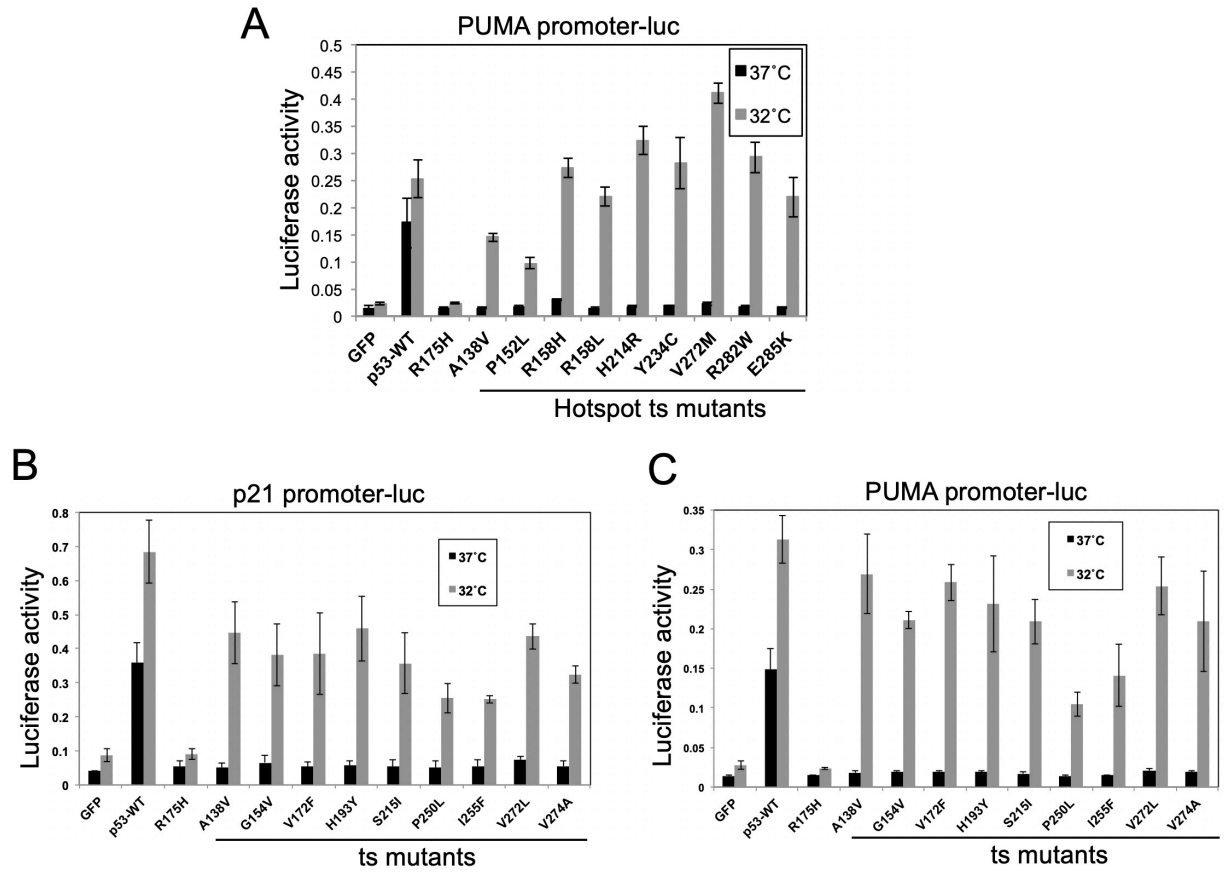

Figure S2. Activation of reporter genes by frequently observed ts p53 mutants. (A, B, C) H1299 cells were transiently transfected with p53 mutants and indicated luciferase reporters for 24 hrs at 37˚C. The cells were kept at 37˚C or shifted to 32˚C for 18 hrs and analyzed for luciferase activity. The results are average of 3 experiments (mean ± SD).

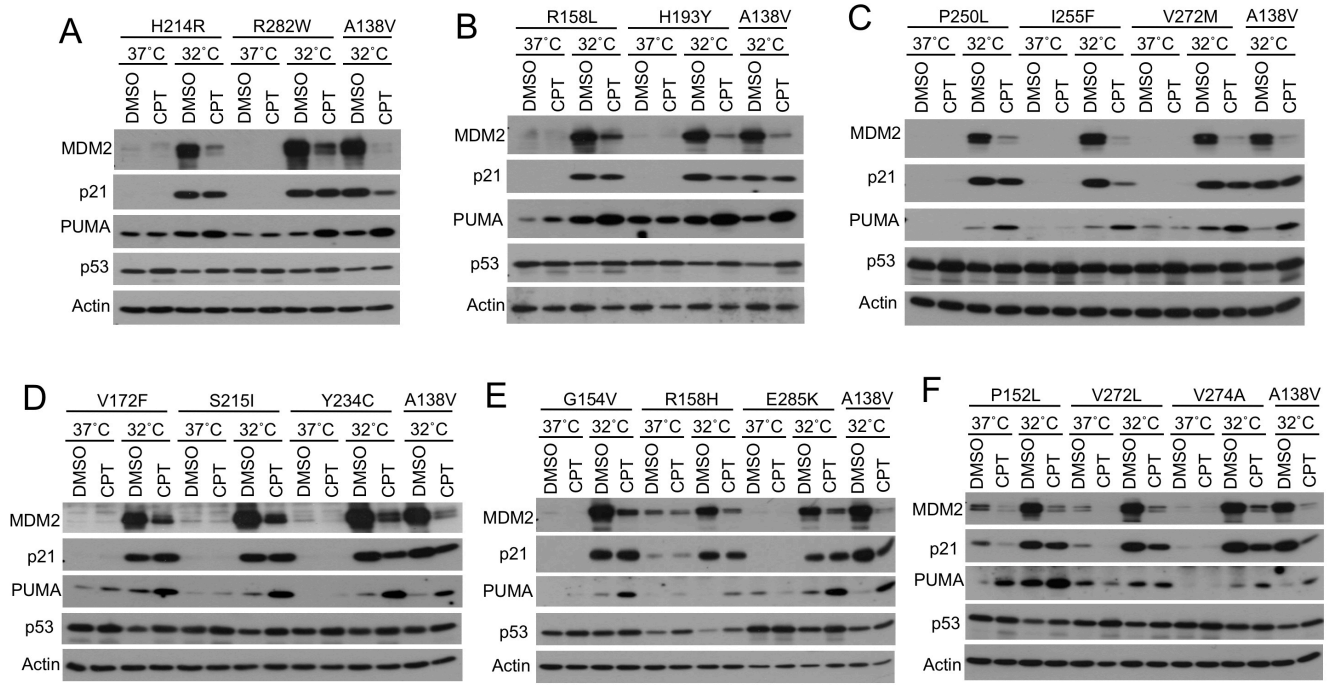

Figure S3. Induction of endogenous targets by ts p53 mutants. (A-F) P53 ts mutants were stably expressed in H1299 cells using lentivirus vector. Cells were tested for induction of target gene expression after 20 hrs at 32˚C by Western blot. CPT (0.5 μM for 20 hrs) was added when cells were shifted to 32˚C. A138V was used as a benchmark to facilitate comparison between gels.

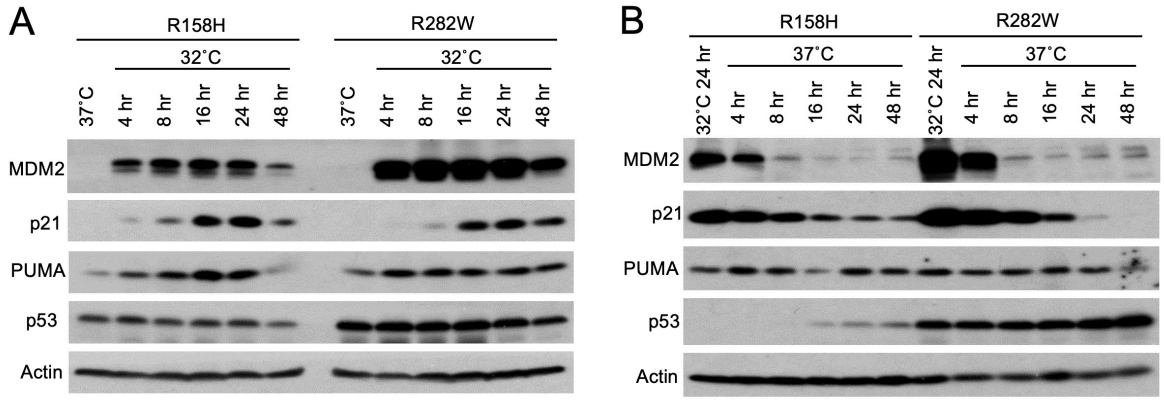

Figure S4. Kinetics of ts p53 activation and inactivation by temperature shift. (A) H1299 cells expressing ts p53 mutants were kept at 37˚C or shifted to 32˚C for indicated durations. P53 activation was analyzed by Western blot. (B) H1299 cells expressing ts p53 mutants were kept at 32˚C for 24 hrs to activate p53, followed by shifting to 37˚C for indicated durations and analyzed by Western blot for down regulation of p53 activity.

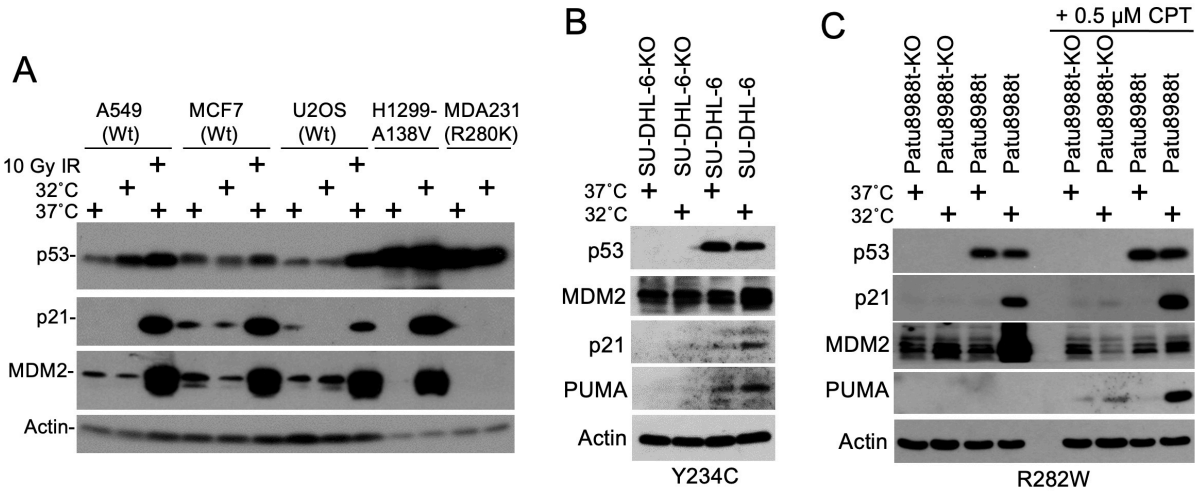

Figure S5. Effect of 32˚C culture on p53 activity. (A) Cell lines with different p53 status [Wt, ts (A138V), non-ts (R280K)] were cultured at 32˚C for 20 hrs, or treated with 10 Gy gamma radiation for 4 hrs. Western blot was performed to determine the induction of p53 target genes. (B, C) Endogenous ts p53 in the cell lines were knocked out using lentivirus expressing Cas9 and p53 gRNA. Clonal cell lines without p53 were analyzed by Western blot after culturing at 37˚C or 32˚C for 18 hrs.

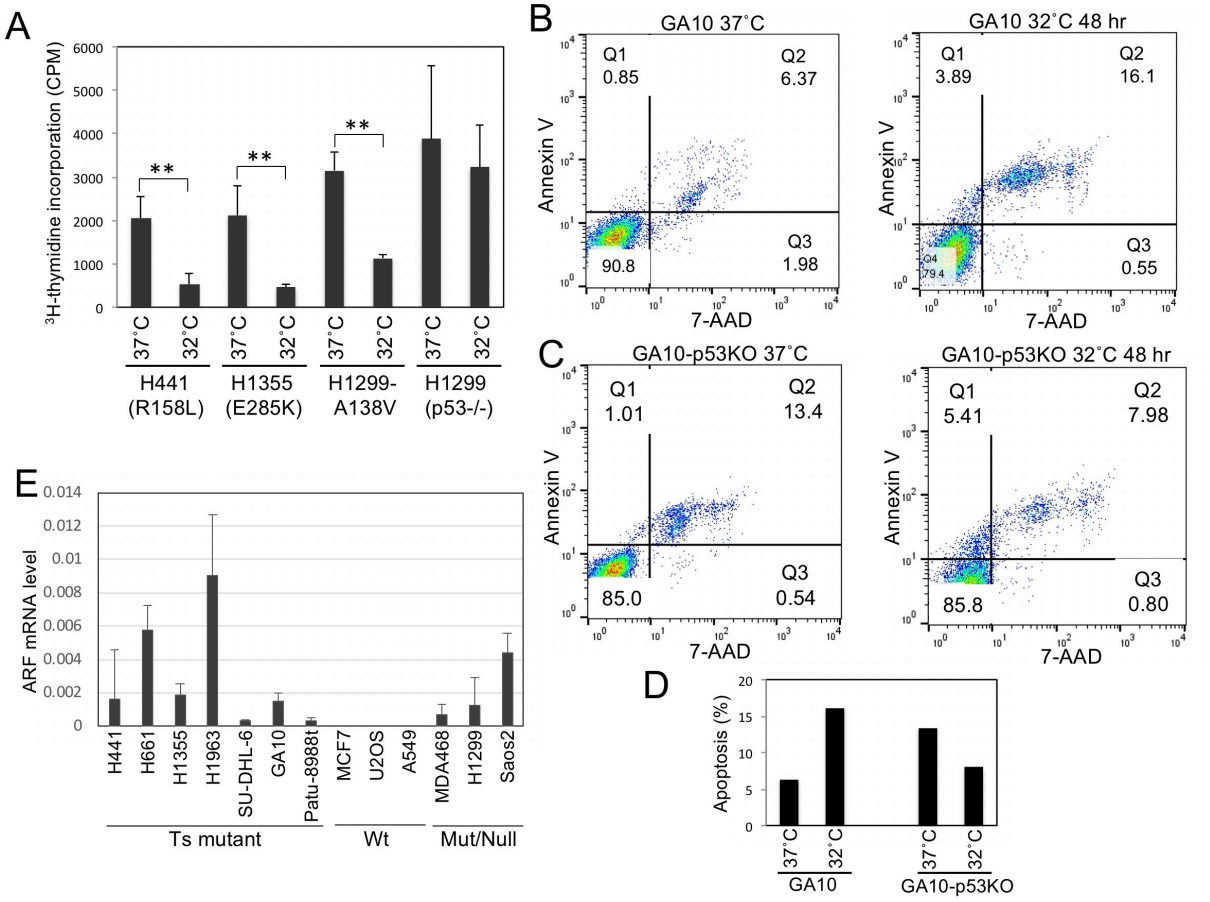

Figure S6. Tumors cells with ts p53 undergo cell cycle arrest and apoptosis at 32˚C. (A) Cells with endogenous ts p53 mutants were shifted from 37˚C to 32˚C for 24 hrs and labeled with ^{3}H-thymidine for 3 hrs. DNA replication rate was measured by scintillation counting. H1299 and H1299 with stable expression of A138V were used as controls. (B, C) GA10 and GA10-p53KO cells were shifted to 32˚C for 48 hrs. Apoptotic cells were identified by Annexin V/7-AAD double staining and FACS analysis and compared to 37˚C controls. (D) Quantification of apoptosis in (B) and (C). (E) ARF mRNA levels in the indicated cell lines was determined using qRT-PCR. The results represent 3 experiments (mean ± SD). **p<0.01.

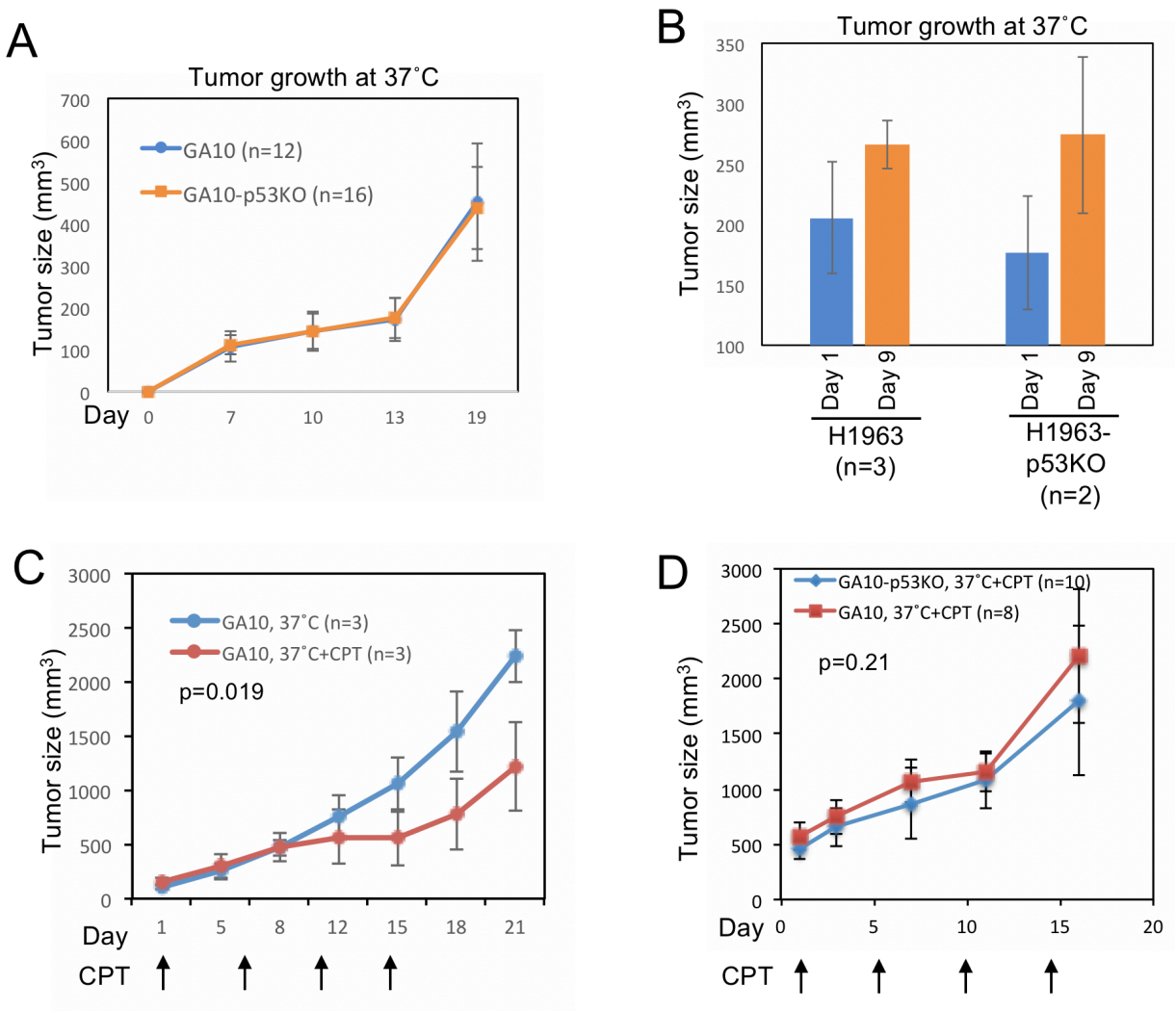

Figure S7. Effect of low-dose CPT treatment alone on tumor growth. (A) Nude mice were inoculated subcutaneously with GA10 and GA10-p53KO cells. Tumor growth at 37˚C (without hypothermia treatment) was determined at indicated time points. (B) Nude mice were inoculated subcutaneously with H1963 and H1963-p53KO cells. Tumor growth at 37˚C (without hypothermia treatment) was determined at indicated time points. (C) Nude mice were inoculated subcutaneously with GA10 cells. When tumors reached ~100 mm^3 the mice were injected i.p. with 1.5 mg/kg CPT every 3 days. Tumor growth was monitored over time (mean ± SD). (D) Nude mice inoculated subcutaneously with GA10 and GA10-p53KO tumors were treated with 1.5 mg/kg CPT at indicated time points without hypothermia. Average size of tumors was plotted over time.

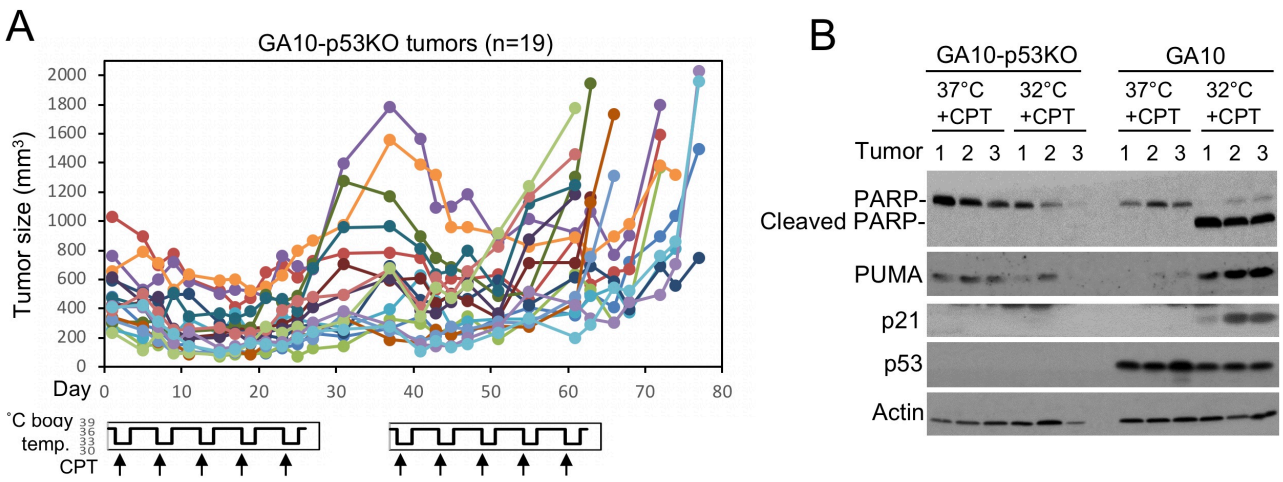

Figure S8. Effects of hypothermia and CPT combination on tumors. (A) Individual growth curves of GA10-p53KO tumors treated with hypothermia+CPT combination, relapsed, and re-treated. (B) Western blot of tumors (n=3) with and without 24 hr hypothermia + CPT treatment.

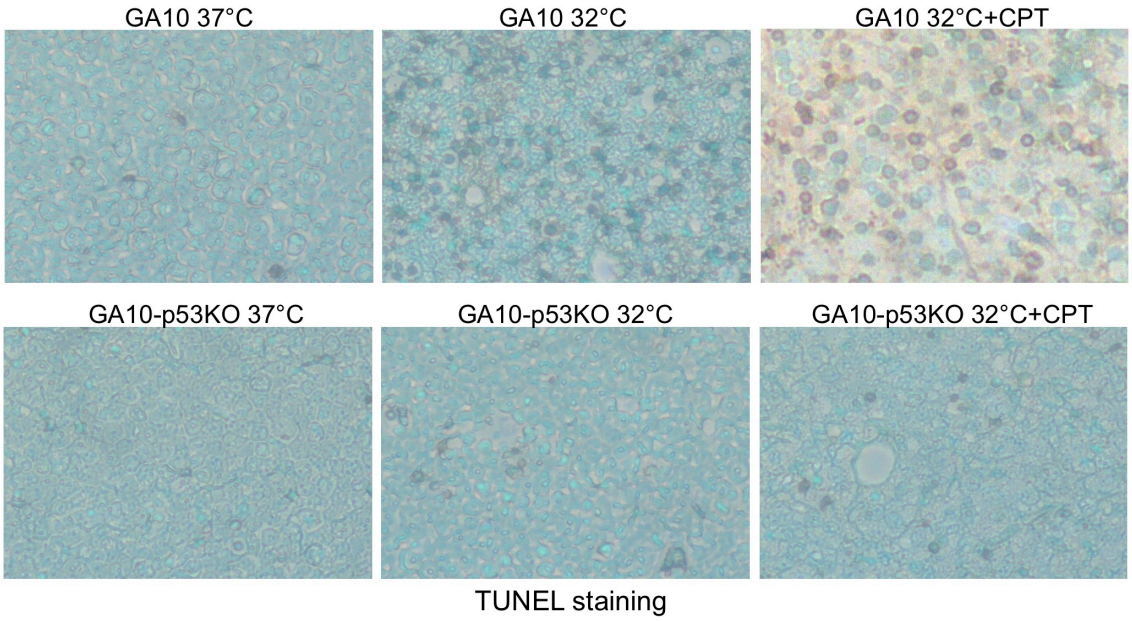

Figure S9. Induction of tumor apoptosis by combination of hypothermia and chemotherapy. Nude mice with tumors were treated for 24 hrs with 32˚C hypothermia and 1.5 mg/kg CPT combination. Tumors were harvested and sections were subjected to TUNEL staining to detect apoptotic cells (brown).