Supporting Information

Lee and Sancar 10.1073/pnas.1106284108

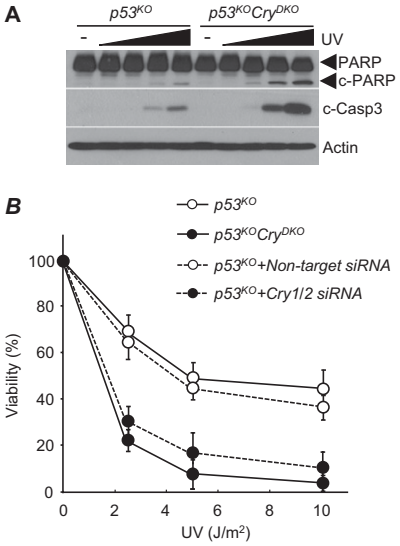

Fig. S1. p53-null and cryptochrome (Cry)-deficient cells are more sensitive to UV-induced apoptosis and clonogenic death than $p53^{KO}$ cells. (*A*) Apoptosis. Unirradiated (–) or irradiated (2, 5, 10, 20 Jm^{-2}) cells of the indicated genotypes were harvested 24 h postirradiation, lysed, and analyzed by immunoblotting. c-Casp3, cleaved caspase 3; c-PARP, cleaved PARP. (*B*) Clonogenic survival. Cells of the indicated genotypes, transfected with the indicated siRNAs for 48 h, were irradiated with the indicated UV dose and then incubated for 9–10 d until colonies were readily visible. Colonies were stained with 5% methylene blue and then counted to obtain the UV survival curves. Results represent the means of three independent experiments (±SD).

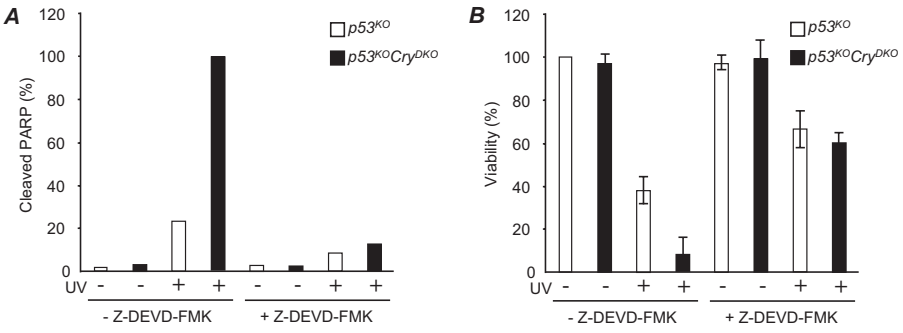

Fig. S2. p73-mediated UV killing of p53-null and Cry-null cells is due to increased apoptosis. Cells of the indicated genotypes were preincubated for 2 h with the caspase-3 inhibitor Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-CH_2F (Z-DEVD-FMK) (R&D Systems) where indicated, irradiated with 10 Jm^{-2}, and analyzed for apoptosis and UV survival. (*A*) Apoptosis. The maximum value of cleaved PARP was set to 100% and other values are plotted relative to the maximum. (*B*) UV survival. A standard clonogenic assay was used to obtain percent survival. Results represent the means (±SD) of three independent experiments.

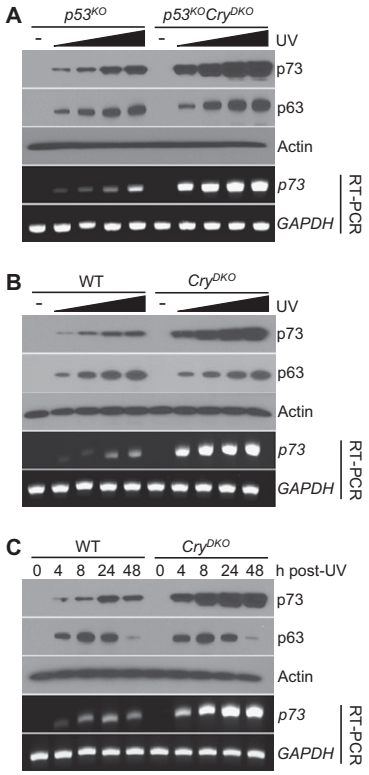

Fig. S3. p73 is highly induced in Cry-null cells by UV irradiation in a time- and dose-dependent manner. (*A* and *B*) Dose–response. Cells of the indicated genotypes were irradiated with 0, 2, 5, 10, or 20 Jm^{-2} and harvested 24 h postirradiation, and p73 induction was probed by immunoblotting or RT-PCR, using actin and *GAPDH* as loading controls. (*C*) Time course. Cells were irradiated with 10 Jm^{-2} and samples were analyzed at the indicated time points.

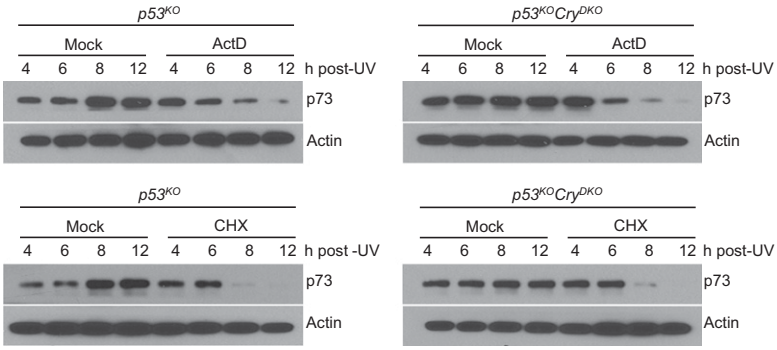

Fig. S4. Cryptochrome disruption does not affect posttranscriptional and posttranslational regulation of p73. Unirradiated or irradiated (10 Jm^{-2}) cells of the indicated genotypes were incubated with either actinomycin D (ActD; 5 μg/mL) or cycloheximide (CHX; 20 μg/mL) 4 h after irradiation, and at the indicated times thereafter samples were analyzed for p73 by immunoblotting.

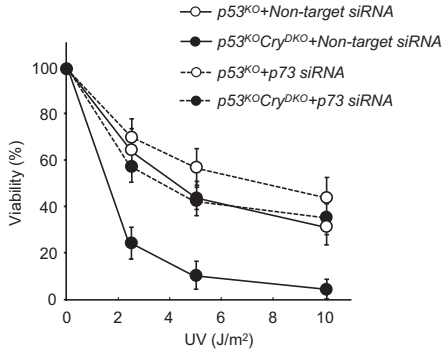

Fig. S5. Down-regulation of p73 in $p53^{KO}Cry^{DKO}$ cells increases their resistance to UV killing. Cells transfected with the indicated siRNAs were irradiated 48 h after transfection, and UV survival was determined by conventional methods. Means (±SD) of three independent experiments are plotted.

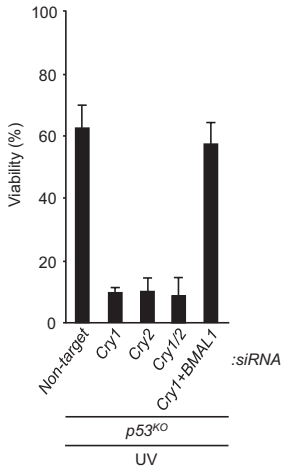

Fig. S6. Core clock proteins Cry and BMAL1 regulate sensitivity of p53-null cells to killing by UV irradiation. *p53*^{KO} cells transfected with the indicated siRNAs were irradiated with 10 Jm^{-2} and survival was determined by the conventional clonogenic assay (±SD, *n* = 3).

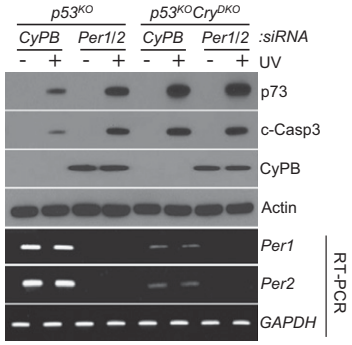

Fig. S7. Down-regulation of Period proteins (Per1 and 2) increases sensitivity of *p53*^{KO} cells to UV-induced apoptosis. Cells transfected with the indicated siRNAs were irradiated with 10 Jm^{-2} where indicated and 24 h later were harvested and analyzed by immunoblotting for protein expression and apoptosis and by RT-PCR for gene expression. Actin and *GAPDH* are internal controls for the immunoblots and RT-PCR reactions, respectively.

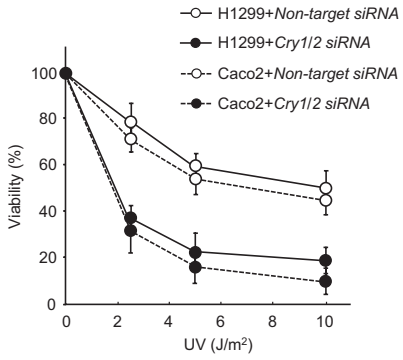

Fig. S8. Cryptochrome disruption increases the sensitivity of human p53-deficient tumor cells to killing by UV irradiation. The clonogenic survival of two human p53-null tumor cell lines without and with cryptochrome down-regulation are shown (±SD, *n* = 3).

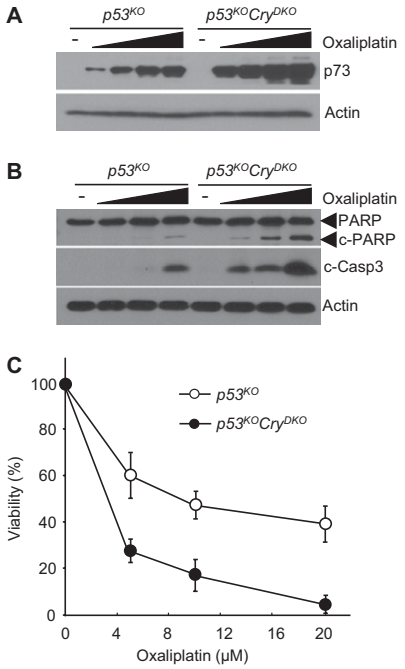

Fig. S9. *p53*^{KO}*Cry*^{DKO} cells are more sensitive to oxaliplatin-induced apoptosis and clonogenic killing than *p53*^{KO} cells. (*A*) Induction of p73 by oxaliplatin. Cells of the indicated genotypes were treated with 0, 2, 5, 10, or 20 μM oxaliplatin and harvested 24 h later, and p73 induction was probed by immunoblotting. (*B*) Cells were treated with 0, 5, 10, or 20 μM oxaliplatin, harvested 24 h later, and analyzed for apoptosis by immunoblotting for PARP and caspase 3. (*C*) Clonogenic survival of oxaliplatin-treated cells (±SD, $n = 3$).

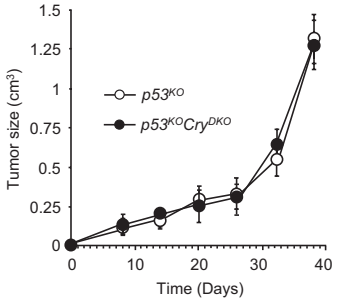

Fig. S10. Growth rates of *p53*^{KO} and *p53*^{KO}*Cry*^{DKO} xenografts in NOD/SCID mice. Tumor xenografts were established by subcutaneous injection of 2×10^6 cells of a *p53*^{KO} genotype in the left flank and a *p53*^{KO}*Cry*^{DKO} genotype in the right flank of a 6-wk-old female mouse. Tumor growth was monitored by a caliper and the size is plotted as a function of time after inoculation. The values are means ± SD ($n = 10$).

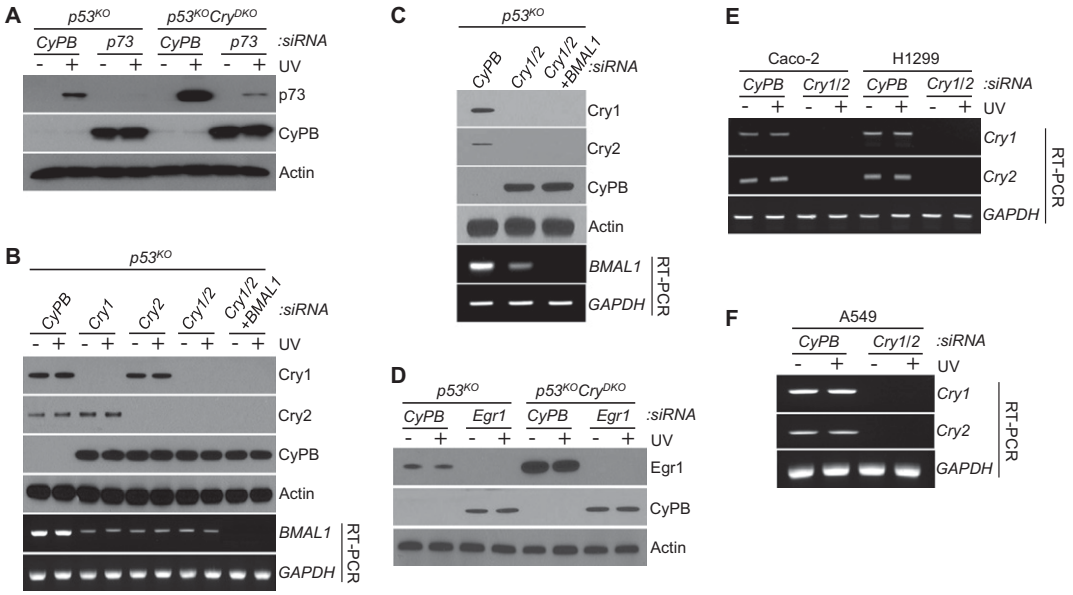

Fig. S11. Specificity and efficiency of siRNAs used to down-regulate apoptosis and clock genes. (*A–D*) Mouse cell lines. (*E* and *F*) Human cell lines. When available, antibodies were used to monitor down-regulation; otherwise, RT-PCR was used for the mRNAs of target genes.

Table S1. Primer sequences for chromatin immunoprecipitation

Gene		Sequence (5′ to 3′)	Protein
Egr1	F	CTCCCTCACTGCGTCTAAGG	BMAL1
	R	CACCCAGAATCGAAAGGCTA	
p73	F	GGACTTTGAAGAGTCCAACC	Egr1
	R	CGCTGCCCTTACTGTCCTAA	
p73	F	CCACTGCCTTTGGAGCTAAG	C-EBPα
	R	GCGAGCTGCAGATTAGAGAC	

F, forward; R, reverse.

Table S2. Primer sequences

Name		Sequence (5′ to 3′)	Species
p73	F	ACCTTCGACACCATGTCTCC	Mouse
	R	GCGAGGTTGTTGCCTTCTAC	
BMAL1	F	CGAAGACAATGAGCCAGACA	Mouse
	R	AAATAGCTGTCGCCCTCTGA	
Per1	F	CTGGCTCCTCCAGTGATAGC	Mouse
	R	CGCTTGGTTGTACTGGGAAT	
Per2	F	AGGATGTGGCAGGTAACAGG	Mouse
	R	TGTACAGTGTGGGGGTGCTA	
GAPDH	F	GGTGAAGGTCGGTGTGAACG	Mouse
	R	CTCGCTCCTGGAAGATGGTG	
Cry1	F	GGCGTTATTTGCCTGTCCTA	Human
	R	ACGTTTCCCACCACTGAGAC	
Cry2	F	GTCCTGCAGTGCTTTCTTCC	Human
	R	CCACACAGGAAGGGACAGAT	
GAPDH	F	ACAGTCAGCCGCATCTTCTT	Human
	R	TTGATTTTGGAGGGATCTCG	