Supporting Information

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SI Methods

Cell Culture and Transfection. HEK 293T and U2OS cells were grown in Dulbecco's modified Eagle's medium, HCT116WT and p53$^{-/-}$ cells were grown in RPMI 1640 medium, and CDK7 (as/as) HCT116 cells were grown in McCoy's 5A medium supplemented with 10% fetal bovine serum at 37 °C. Doxorubicin and Cisplatin were from Sigma, Nutlin3 was from Cayman Chemical, and 3MB-PPI was from EMD chemicals. Transfection of plasmids was performed using Effectene reagent (Qiagen). Cells were harvested 48 h after transfection and processed for different assays. pSUPER driven expression of TFIIB shRNA was performed by the double transfection method described previously (1). The siRNAs were obtained from Qiagen, and transfection was performed by using Hiperfect (Qiagen) for 48 h.

Luciferase Assay and Western Blotting. The AREG and p21 FL promoter luciferase constructs have been described before (2). The core promoter constructs contained nucleotides −56 to +4 of AREG and −56 to +3 of the p21 promoters cloned downstream of 5-GAL4 DNA binding sites. The AREG-p53bs luciferase construct was generated using 4-tandem upstream p53-binding sequences (5'-CCAGGCAAGTCCAGGCAGG-3') cloned between Nhe1 and Xho1 restriction enzyme sites of the AREG promoter region. Luciferase activity was measured with the Promega Luciferase kit. Western blotting analysis was performed as described before (3) using antibodies listed in Table S1.

ChIP Assay. RNA extraction and quantitative RT-PCR analysis was performed as described previously (3). ChIP assay was performed with HEK293T, HCT116WT, and HCT116 p53$^{-/-}$ cells as described previously (3). Statistical significance was analyzed by Student's t test ($P < 0.05$). The sequences of the qPCR primers used in the study are listed in Table S2.

Kinase Assay. A kinase assay with a panel of different CDK/cyclins was performed at Reaction Biology Corp. (Malvern, PA) using HotSpot 33P-ATP Filter-binding kinase assay format. The activity of the kinases was normalized under standard conditions using control substrates, and subsequently their activity on TFIIB was tested at different time points (30, 60, 120, and 180 min) at room temperature.

1. Elsby LM, O'Donnell AJ, Green LM, Sharrocks AD, Roberts SG (2006) Assembly of transcription factor IIB at a promoter in vivo requires contact with RNA polymerase II. *EMBO Rep* 7(9):898–903.
2. Deng W, Malecová B, Oelgeschläger T, Roberts SG (2009) TFIIB recognition elements control the TFIIA-NC2 axis in transcriptional regulation. *Mol Cell Biol* 29(6):1389–1400.
3. Wang Y, Fairley JA, Roberts SG (2010) Phosphorylation of TFIIB links transcription initiation and termination. *Curr Biol* 20(6):548–553.

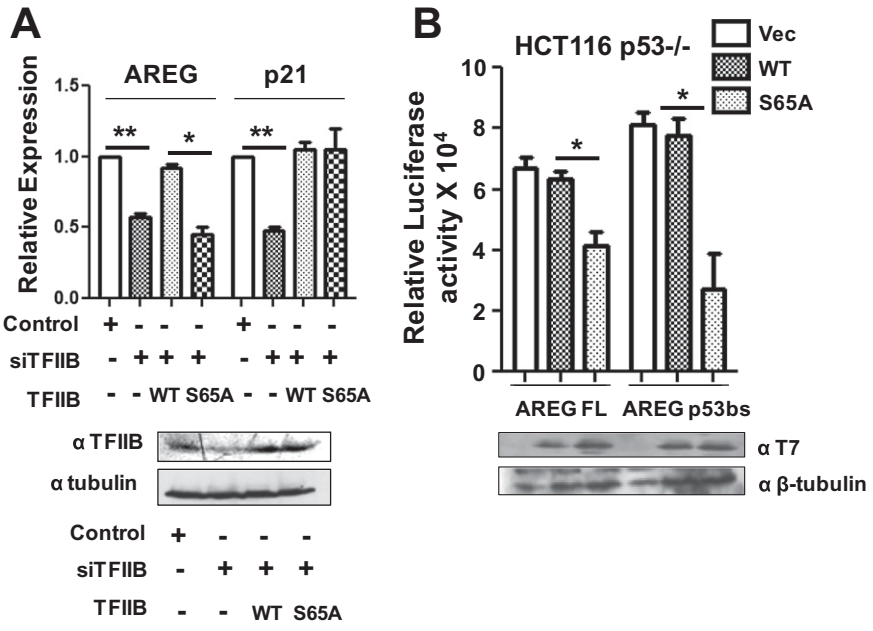

Fig. S1. p53 can bypass the requirement for the integrity of TFIIB Ser65. (*A*) The endogenous expression of AREG and p21 was determined by qPCR in HCT116WT cells transfected with pSUPER shTFIIB along with WT or S65A TFIIB. Transfection with empty pSUPER vector and shTFIIB are controls. Error bars denote SD. Western blotting analysis of the transfected cells was performed with anti-TFIIB and anti-β tubulin antibodies. (*B*) Luciferase assay was performed with AREG promoter luciferase construct under the control of four tandem upstream p53-binding sites (AREG p53bs) and was compared with the original AREG FL promoter in the presence of wild-type TFIIB and mutant S65A in HCT116 p53-null cells. The expression of the WT and S65A TFIIB proteins was analyzed by immunoblotting with anti-T7 and anti-β tubulin antibodies (*Lower*).

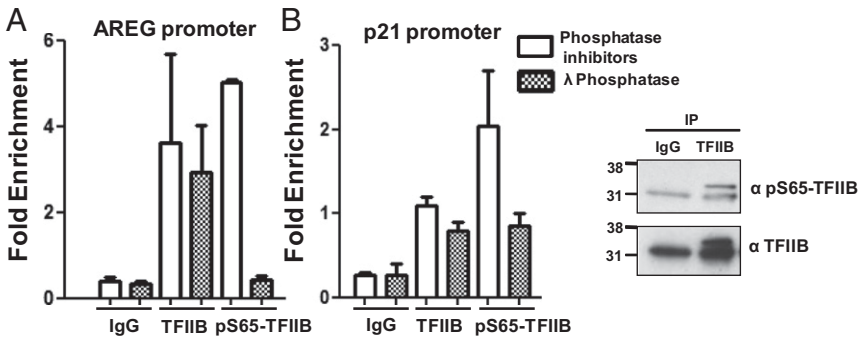

Fig. S2. TFIIB present at the p21 promoter contains phosphoserine-65. (*A*) ChIP assay was performed with anti-TFIIB and pSer65-TFIIB antibodies in presence of either phosphatase inhibitor mixture or after lambda phosphatase treatment to determine the relative occupancy of phosphorylated-ser65-TFIIB at the promoters of the AREG and (*B*) the p21 gene. qPCR data of three independent experiments are plotted as fold enrichment relative to 18S DNA. Error bars denote SD. Western blotting analysis was performed with TFIIB-immunoprecipitates using anti-pS65-TFIIB and TFIIB antibodies.

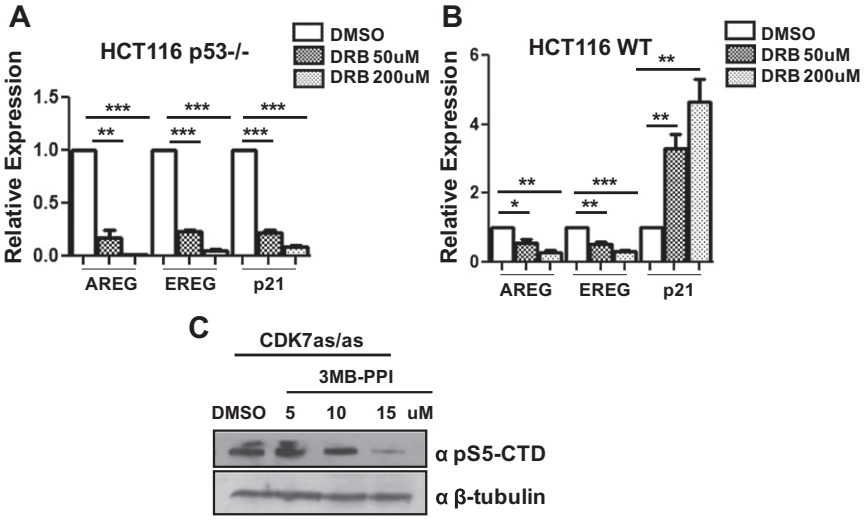

Fig. S3. CDK7 phosphorylates TFIIB. (*A* and *B*) qPCR analysis of AREG and p21 mRNA after treatment with the indicated concentrations of DRB in HCT116 $p53^{-/-}$ (*Left*) and WT cells (*Right*) for 24 h. The values are normalized relative to the expression of 18S rRNA. (*C*) CDK7 (as/as) cells were treated with the indicated concentrations of 3MB-PPI for 12 h and immunoblotted with phospho-ser5-CTD (of RNAPII) and β-tubulin antibodies.

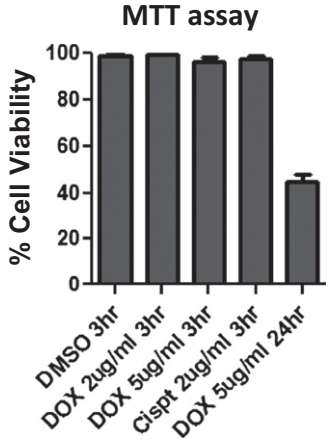

Fig. S4. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to estimate the cell viability after indicted time points and concentrations of doxorubicin and cisplatin treatment in HCT116 WT cells.

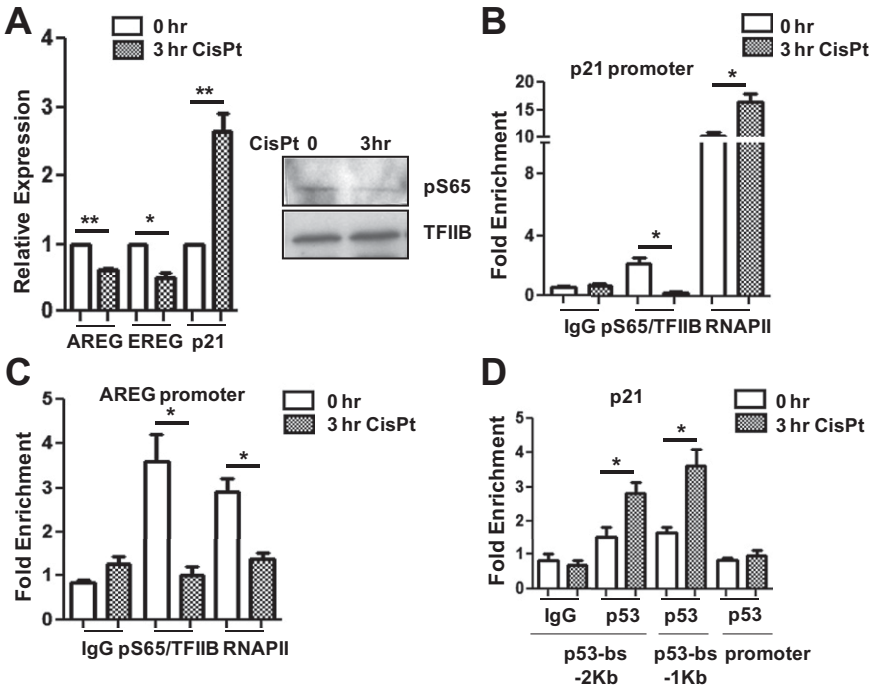

Fig. S5. The level of phospho-TFIIB Ser65 is reduced upon treatment with DNA-damaging agents. (*A*) qPCR analysis of AREG, EREG, and p21 gene expression was performed after 3 h of 2 μg/mL cisplatin treatment in HCT116 WT cells. TFIIB was immunoprecipitated from HCT116 WT cells after 3 h of 2 μg/mL cisplatin treatment and immunoblotted using anti-pS65-TFIIB and anti-TFIIB antibodies. (*B*) ChIP analysis was carried out with anti-TFIIB, anti-pS65-TFIIB, and anti-RNAPII antibodies in HCT116 WT cells treated with 2 μg/mL cisplatin after 3 h. The normalized level of pS65 over TFIIB and RNAPII at the p21 and (*C*) AREG promoters is plotted as fold enrichment relative to 18S DNA. Error bars denote SD. (*D*) Similar ChIP analysis was carried out with anti-p53 antibodies at different regions of the p21 promoter.

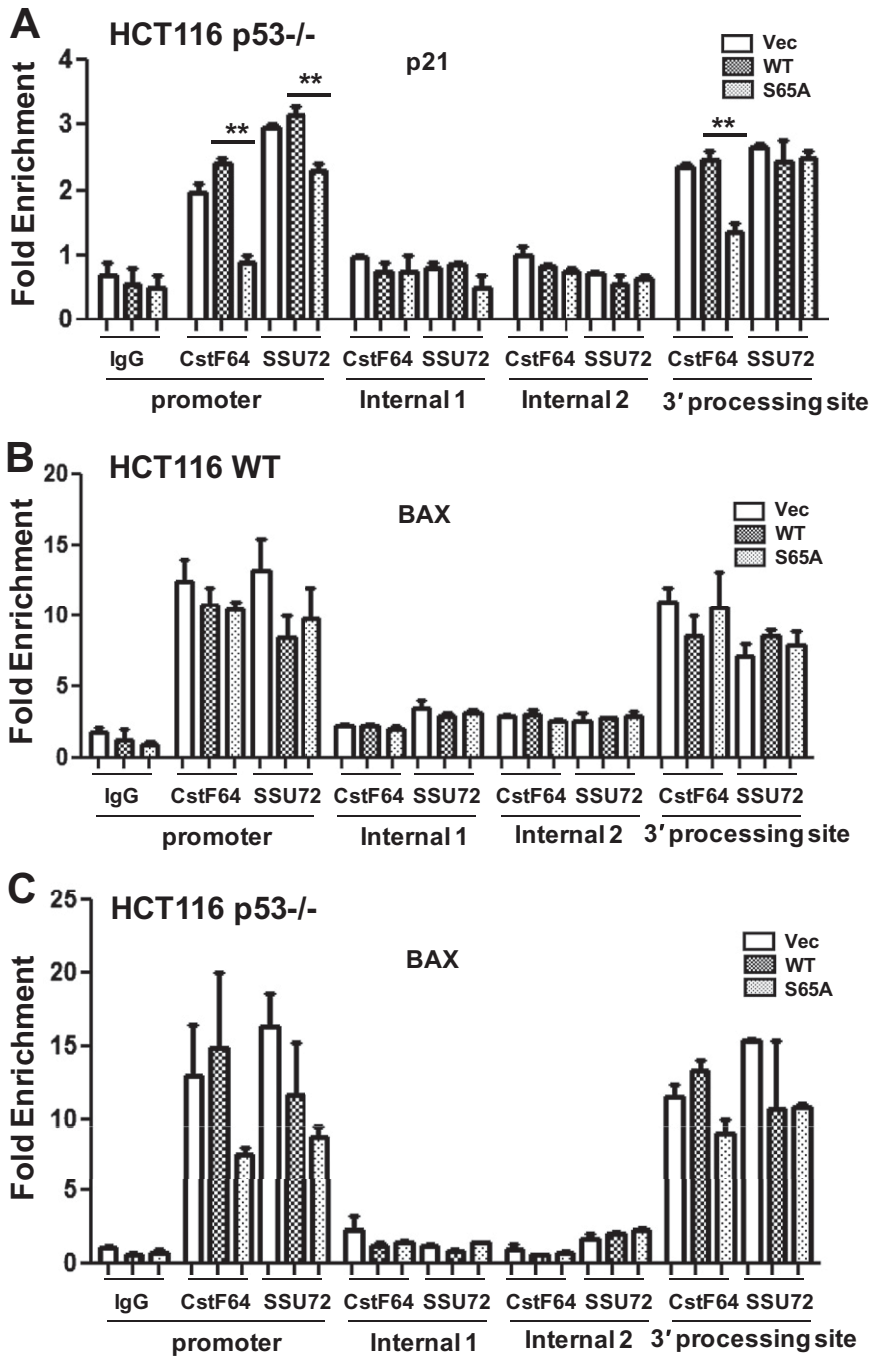

Fig. S6. p53-target genes bypass TFIIB Ser-65 phosphorylation for recruitment of 3′-processing factors. (*A*) ChIP assays were performed to determine the recruitment of CstF-64 and SSU72 to the promoter and 3′-processing regions of the p21 gene in HCT116 $^{-/-}$ cells that had been transfected with either pCDNA3 vector, or the same vector driving expression of wild-type TFIIB or TFIIB S65A. (*B*) Similar ChIP assays were performed for BAX gene in HCT116 WT and (*C*) p53$^{-/-}$ cells. Two internal regions (1 and 2) of the p21 and BAX genes were analyzed for any nonspecific enrichment in CstF-64 and SSU72 ChIP DNA. qPCR data of three independent transfections are plotted as fold enrichment relative to 18S DNA. Error bars denote SD.

Table S1. Antibodies used in ChIP assay and Western blotting

Antibody	Catalog no.	Company
T7	69522–3	Novagen
β-tubulin	ab6046	Abcam
Ssu72	sc-69613	Santa cruz
CstF-64	sc-28201	Santa cruz
p53 (DO1)	sc-126	Santa cruz
CDK7	sc-7344	Santa cruz
CDK9	sc-484	Santa cruz
RNAPII	ab5408	Abcam
p-Ser5-CTD RNAPII	ab5131	Abcam
p-Ser65-TFIIB		made by Eurogentec
TFIIB		made by Eurogentec
Secondary antibodies		Jackson Immuno Research

Table S2. Primer sequences

Gene	Primer sequences
5′—3′ qRT-PCR primer	
Amphiregulin	Fwd: AGAGTTGAACAGGTAGTTAAGCCC
	Rev: GTCGAAGTTTCTTTCGTTCCTCAG
γ-actin	Fwd: GTTTGAGACCTTCAACACCC
	Rev: CTTCATGAGGTAGTCGGTCAG
EREG	Fwd: GCTCTGCCTGGGTTTCCATC
	Rev: CCACACGTGGATTGTCTTCTGTC
Bcl2	Fwd: TCCGCATCAGGAAGGCTAGA
	Rev: AGGACCAGGCCTCCAAGCT
p21	Fwd: GCAGACCAGCATGACAGATTT
	Rev: GGATTAGGGCTTCCTCTTGGA
BAX	Fwd: GCCCTTTTGCTTCAGGGTTT
	Rev: TCCAATGTCCAGCCCATGAT
PUMA	Fwd: ACGACCTCAACGCACAGTACG
	Rev: TCCCATGATGAGATTGTACAGGAC
GADD45A	Fwd: TCAGCGCACGATCACTGTC
	Rev: CCAGCAGGCACAACACCAC
MDM2	Fwd: GGTGAGGAGCAGGCAAATGTGCAA
	Rev: ACCAGGGTCTCTTGTTCCGAAGC
DR5	Fwd: TGTCGCCGCGGTCCT
	Rev: TGGGTGATCAGAGCAGACTCAG
18S	Fwd: GTAACCCGTTGAACCCCATT
	Rev: CCATCCAATCGGTAGTAGCG
5′—3′ ChIP primer	
AREG promoter	Fwd: TCCACTTCCTCTCAGCGAAT
	Rev: GGTGTGCGAACGTCTGTAGG
AREG terminator	Fwd: CATAAGACAATGGACCCTTTTTG
	Rev: AATACTTTTTACCTTCGTGCACCT
AREG Internal 1	Fwd: GTATCCCGGCGAGAGG
	Rev: CCAGAGTTAAGCCACTGC
AREG Internal 2	Fwd: GCCAATTTGGCGGGA
	Rev: GCAAATTCAGCTGTCCC
p53-bs-2Kb	Fwd: AGCAGGCTGTGGCTCTGATT
	Rev: CAAAATAGCCACCAGCCTCTTCT
p53-bs-1Kb	Fwd: CTGTCCTCCCCGAGGTCA
	Rev: ACATCTCAGGCTGCTCAGAGTCT
p21 promoter	Fwd: TATATCAGGGCCGCGCTG
	Rev: GGCTCCACAAGGAACTGACTTC
p21 terminator	Fwd: CCTCCCACAATGCTGAATATACAG
	Rev: AGTCACTAAGAATCATTTATTGAGCACC
p21 Internal 1	Fwd: GCACCATCCTGGACTCAAGTAGT
	Rev: CGGTTACTTGGGAGGCTGAA
p21 Internal 2	Fwd: AGTCACTCAGCCCTGGAGTCAA
	Rev: GGAGAGTGAGTTTGCCCATGA
BAX promoter	Fwd: GATCCAGGTCTCTGCAA
	Rev: CCCCGCAAAATGGCC
BAX terminator	Fwd: GATTAGTGCCTTCTGCCC
	Rev: GGTCAGAGGGTCATCAATG
BAX Internal 1	Fwd: CTGACGGCAACTTCAAC
	Rev: GGTGCACAGGGCCTT
BAX Internal 2	Fwd: CACCACCGCGCCTCA
	Rev: GGGCAGGGACGGTGG
18S gene	Fwd: GTAACCCGTTGAACCCCATT
	Rev: CCATCCAATCGGTAGTAGCG