E2F1 Functions as an Accessibility Factor for DNA Repair

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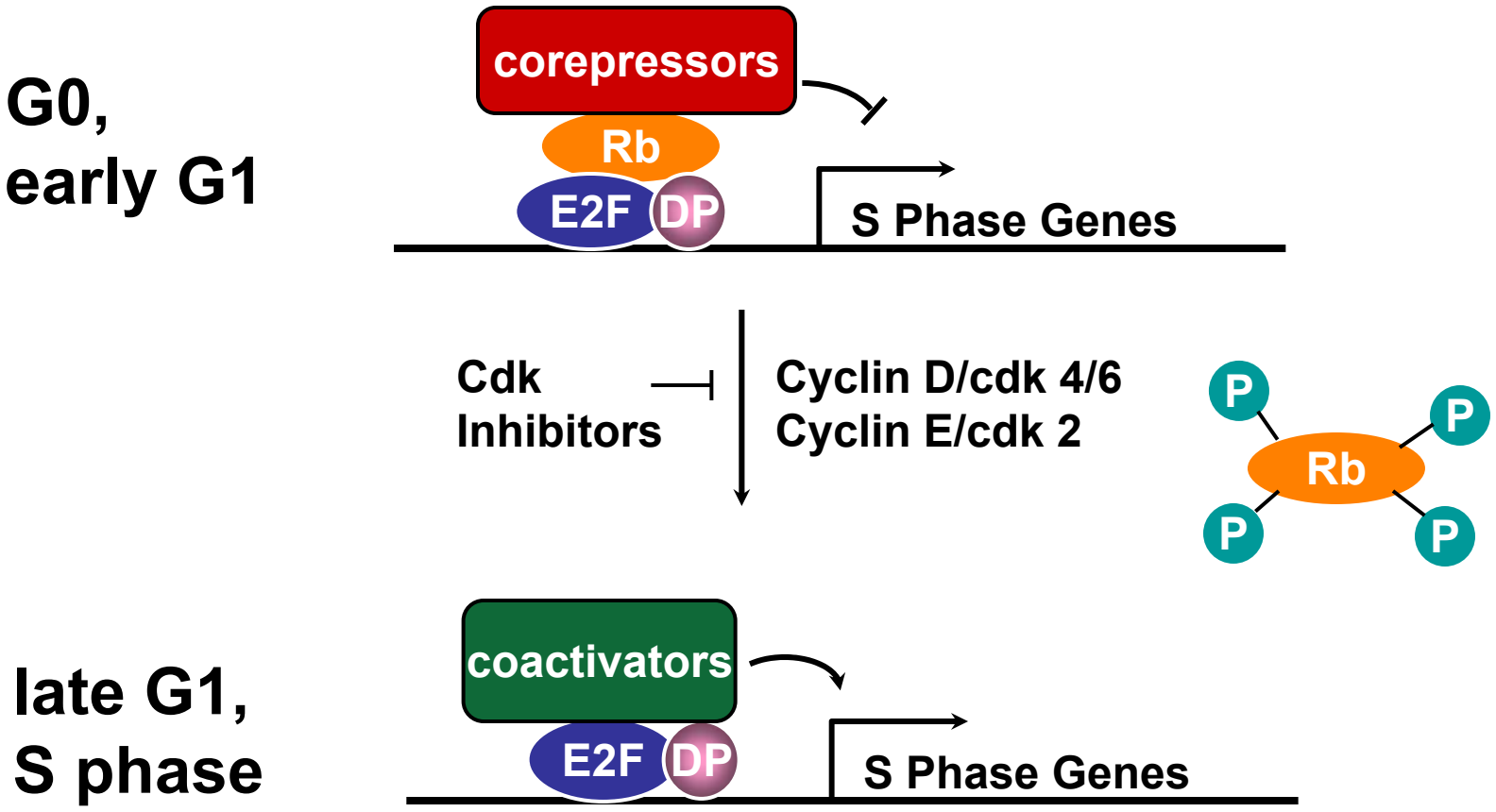
Regulation of E2F Transcriptional Activity

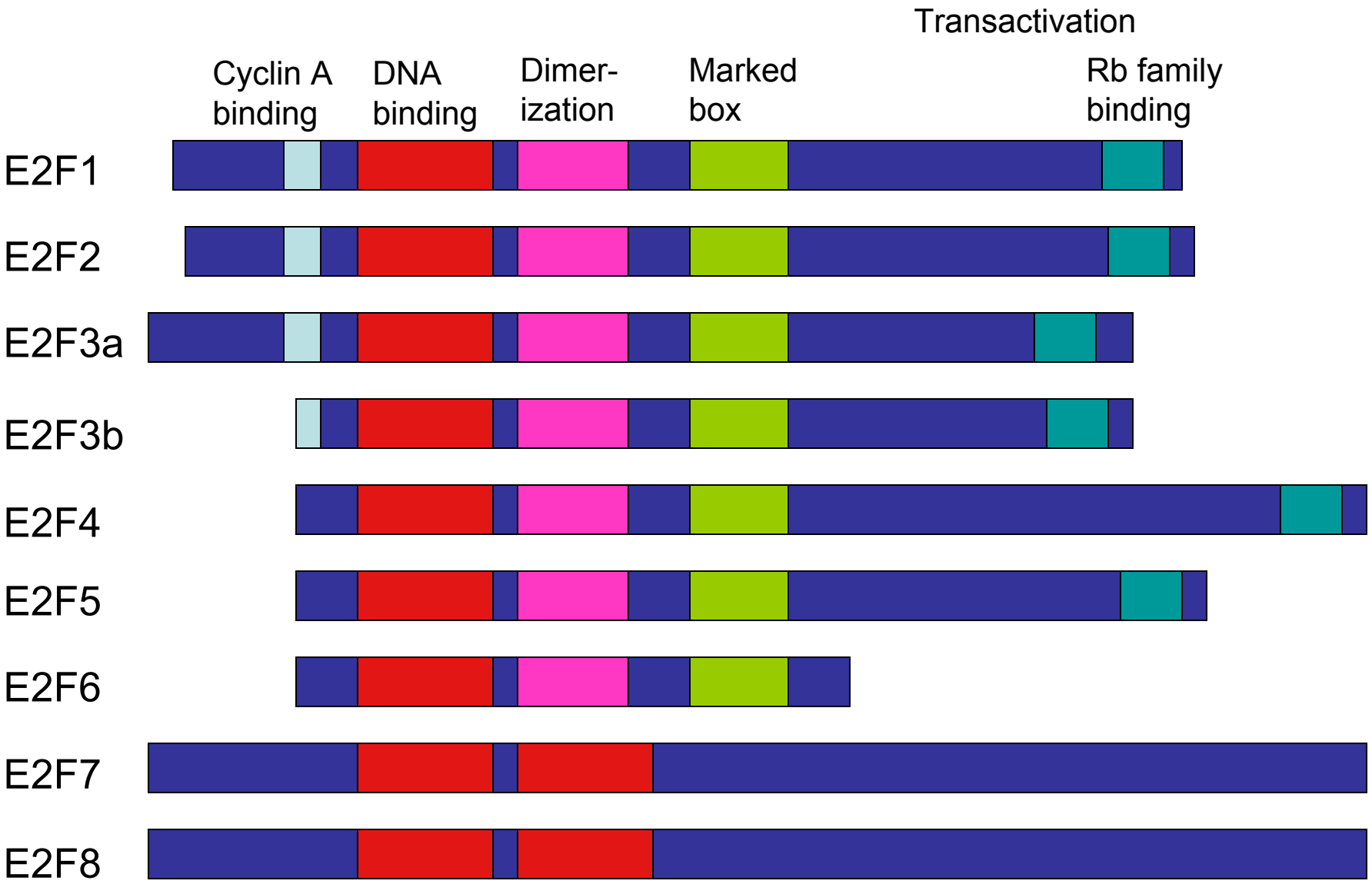


E2F Target Genes

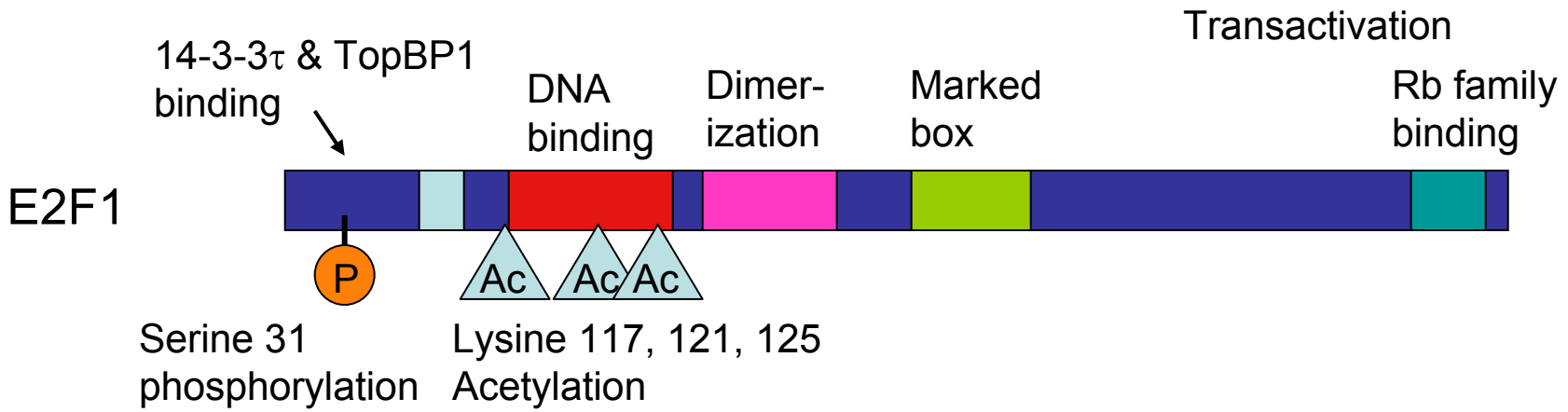
DNA Replication	Cell Cycle Regulation	Transcription Factors
DNA polymerase α	Cyclins A, B, E	E2F1, 2, 3a
PCNA	Cdk1, 2	c-myc
Replication protein A	Cdc25	c-myb
Replication factor C4	Bub1	B-myb
Cdc6	p107	NFATc1, c3
Mcm2, 3, 4, 5, 6, 7	p18INK4c	HMG2, 4
Orc1	p19INK4d	Homeobox A10, 7, 9
Topoisomerase	p19ARF	Enhancer of zeste

Nucleotide Biosynthesis	Apoptosis	DNA repair
Ribonucleotide reductase	p73	Rad51
Thymidine kinase	Apaf1	BRCA1
DHFR	Caspase 3, 7	Uracil DNA gylcosylase
Thymidylate synthase	ASK1	MSH2, 6
Deoxycytidine kinase	AMPKα2	DNA polymerase δ

The E2F Family



E2F1 Responds to DNA damage



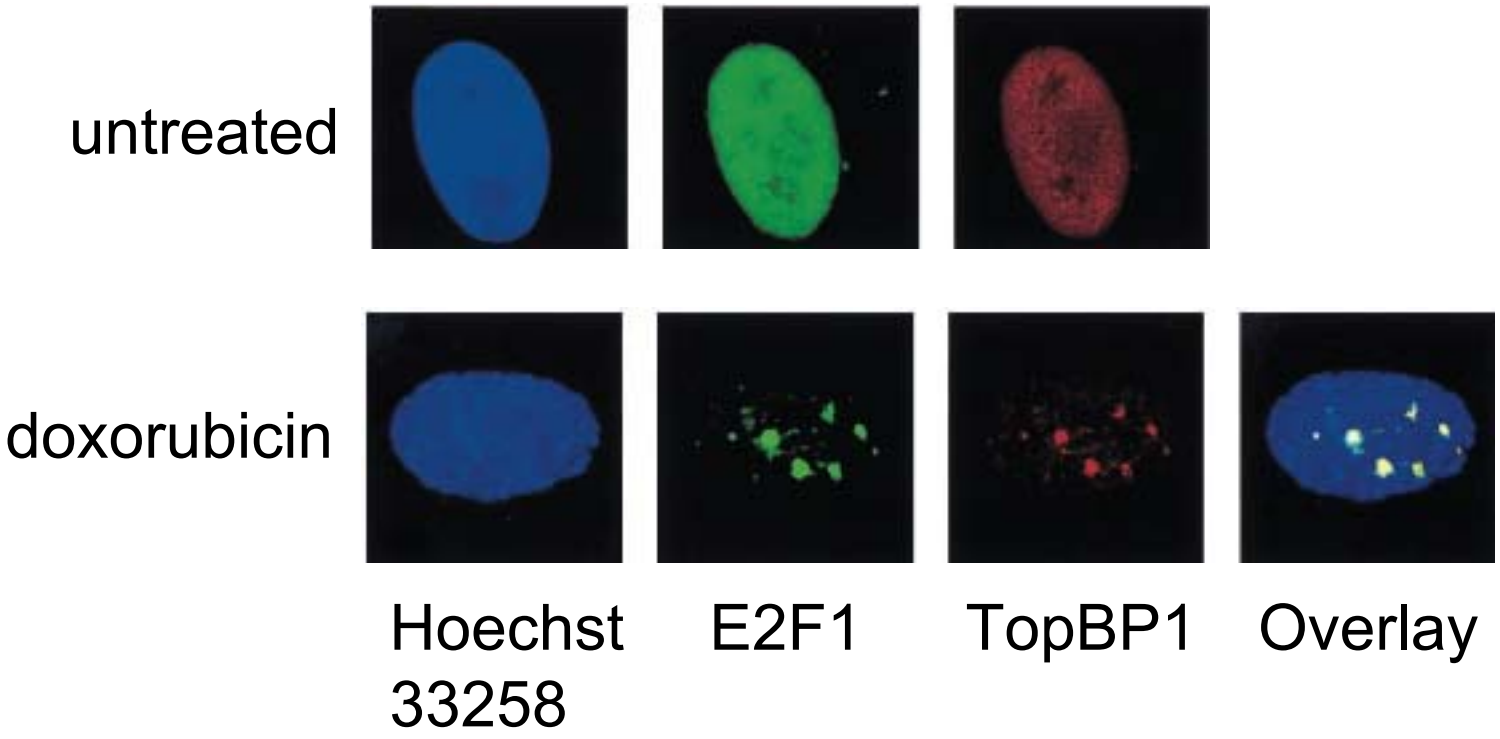
1) E2F1 is unique among the E2F family in that it is stabilized in response to a variety of DNA damaging agents, much like p53 (Blattner, 1999; Hofferer, 1999).

2) This involves phosphorylation of E2F1 at serine 31 by the ATM or ATR kinases and E2F1 binding to 14-3-3τ (Lin, 2001; Wang, 2004).

3) E2F1 is also acetylated in response to double-strand breaks, but not UV-induced damage, and this is associated with E2F1-mediated induction of p73 gene expression and the promotion of apoptosis (Pediconi, 2003; Ianari, 2004).

4) Serine 31 phosphorylation also creates a binding site for one of the BRCT domains in TopBP1. This represses E2F1's transcriptional activity independent of Rb and suppresses E2F1-mediated apoptosis (Liu 2004; Liu, 2004).

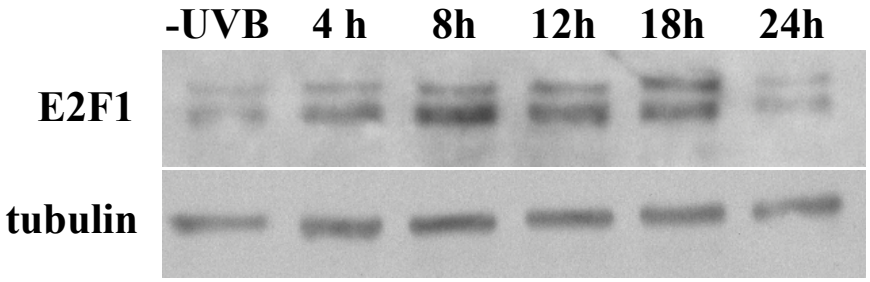
Binding to TopBP1 also “Sequesters” E2F1 at sites of DNA damage

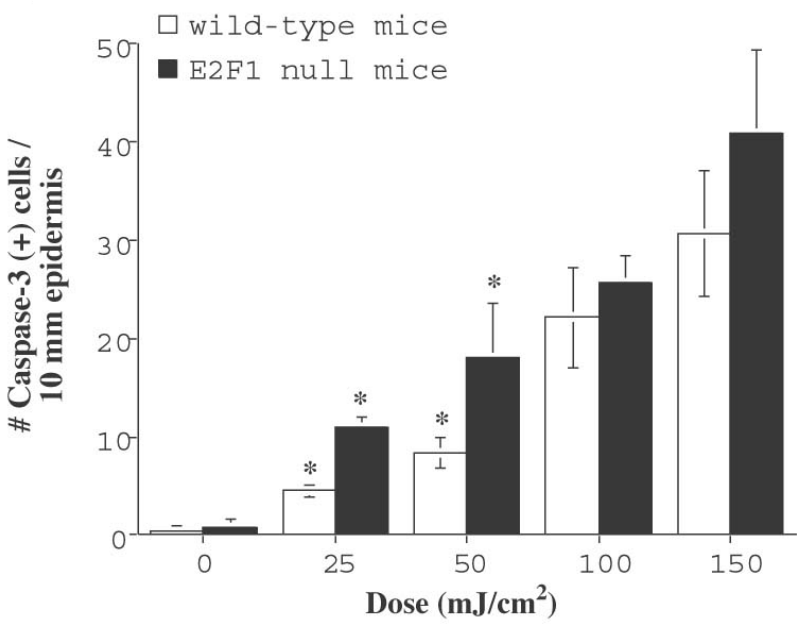


Lui et al,. 2004

Regulation of epidermal apoptosis and DNA repair by E2F1 in response to ultraviolet B radiation

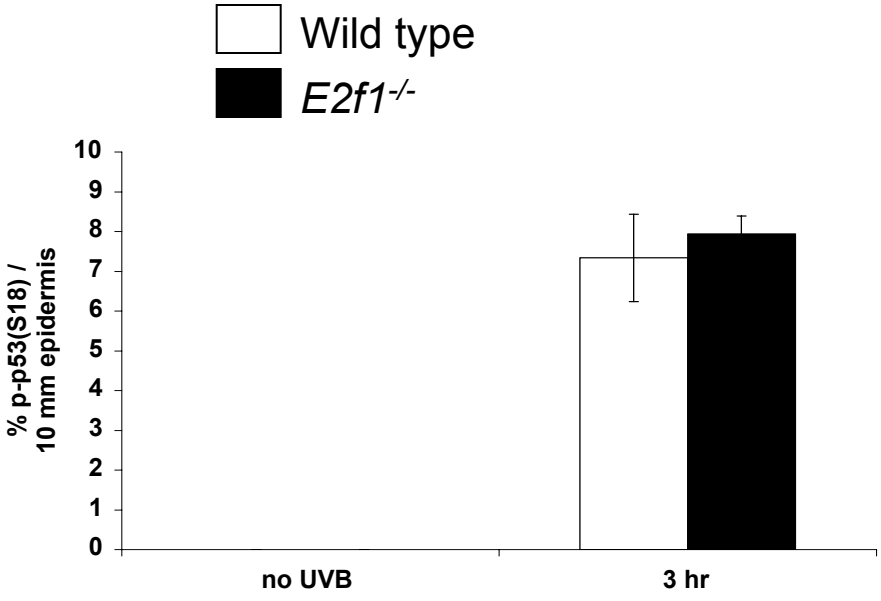
Thomas R Berton[1], David L Mitchell[1], Ruifeng Guo[1] and David G Johnson*,[1]

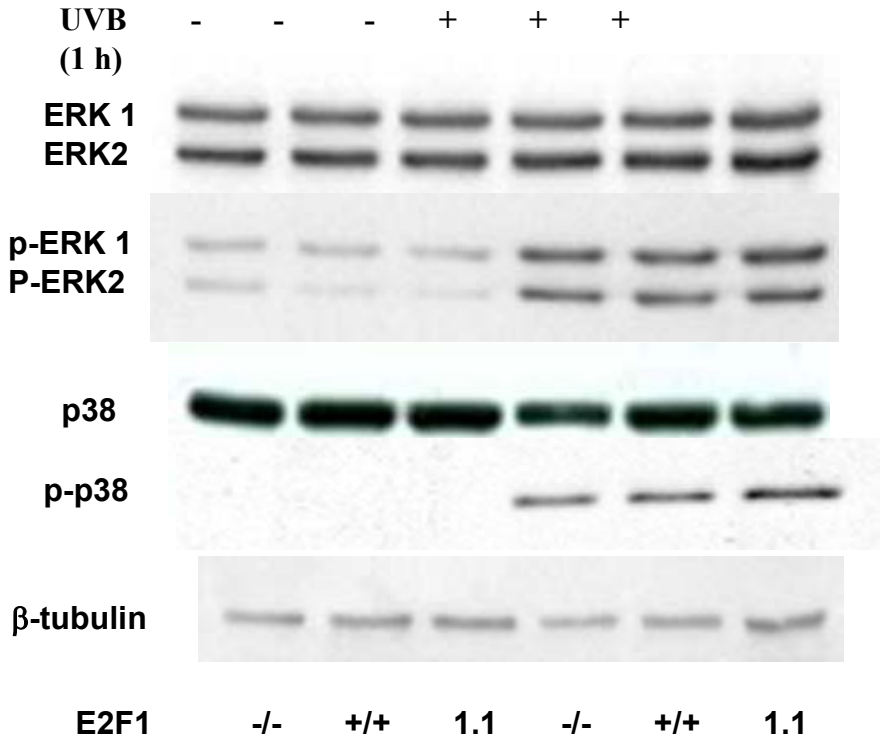




E2F1 Suppresses UVB-Induced apoptosis

E2f1 Status Does not Affect p53 Induction or Activation of MAP Kinases in Response to UV





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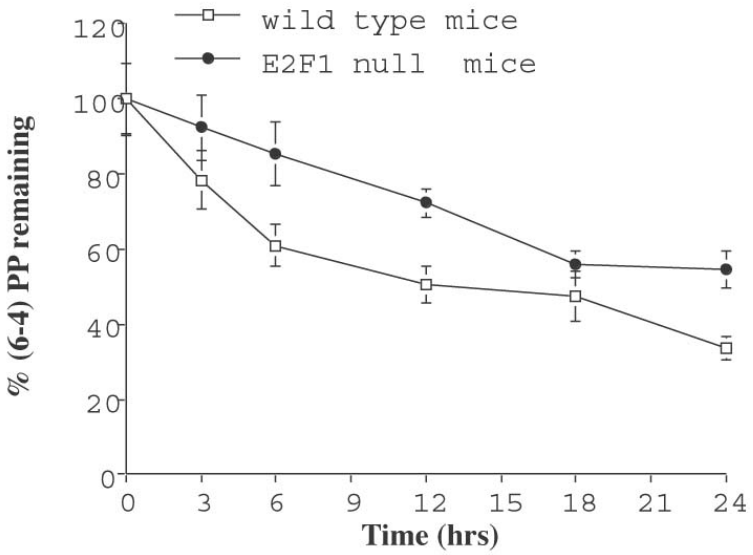
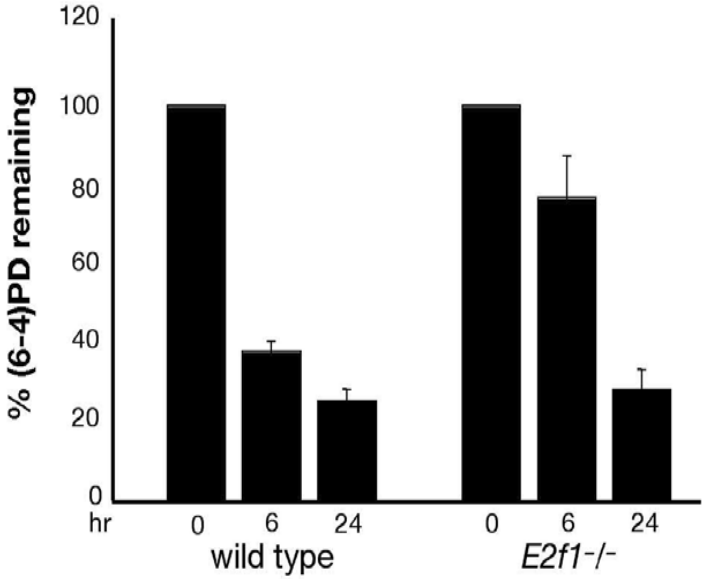
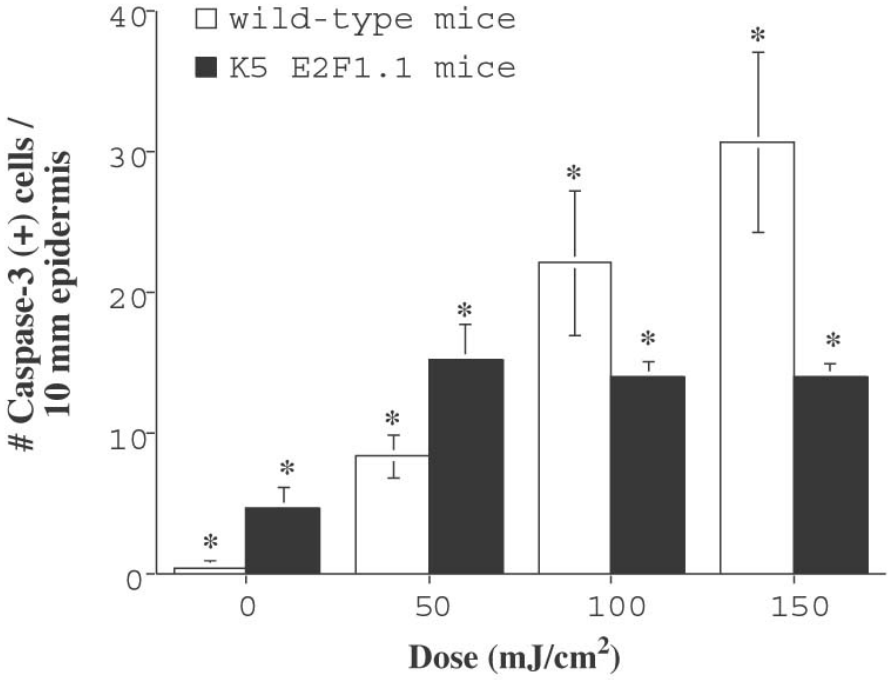
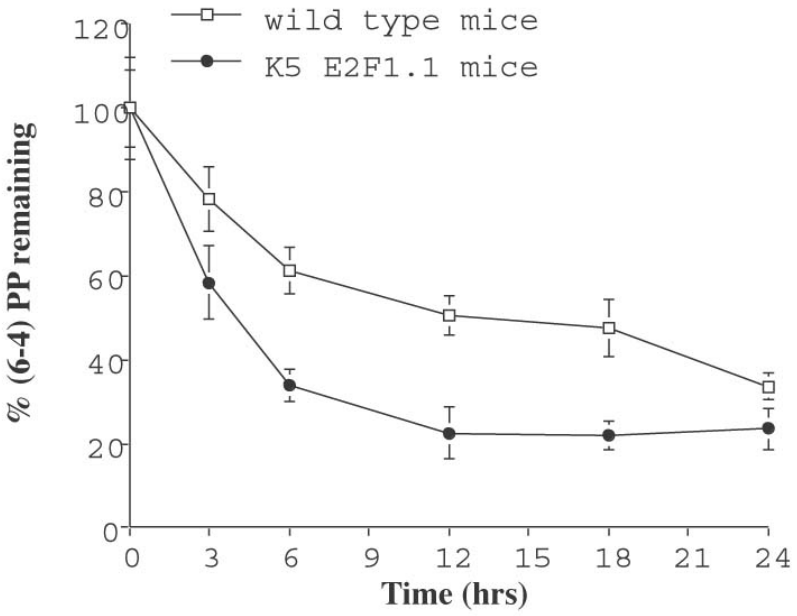




Inactivation of *E2f1* Reduces DNA Repair Efficiency

Transgenic Expression of E2F1 Stimulates DNA Repair and Suppresses Apoptosis Following UV Irradiation



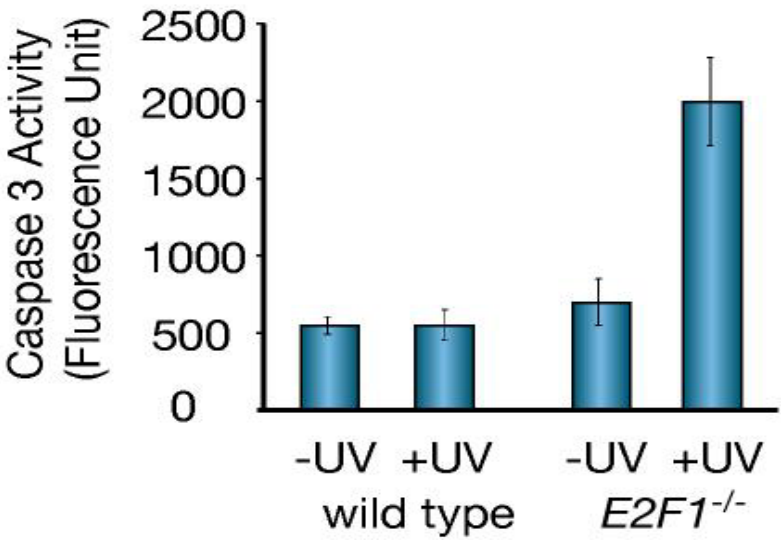


Acknowledgements

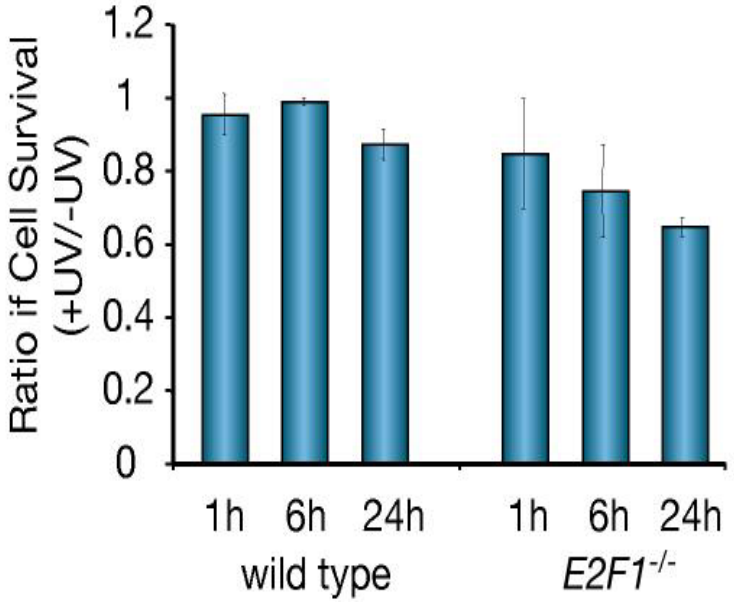
Ruifeng (Ray) Gou and Jie (Claire) Chen

E2F1 Suppresses Apoptosis and Promotes Cell Survival in Response to UV Irradiation

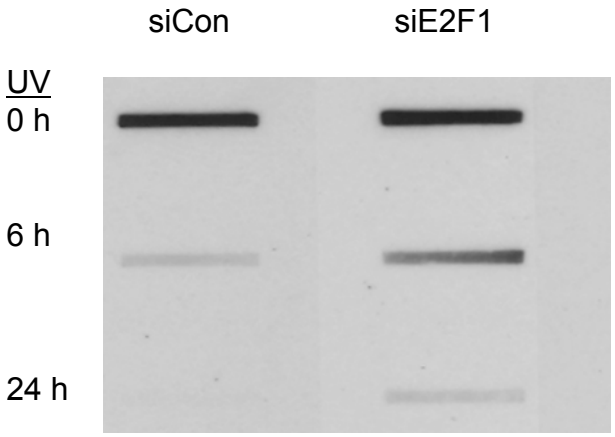
Apoptosis Assay



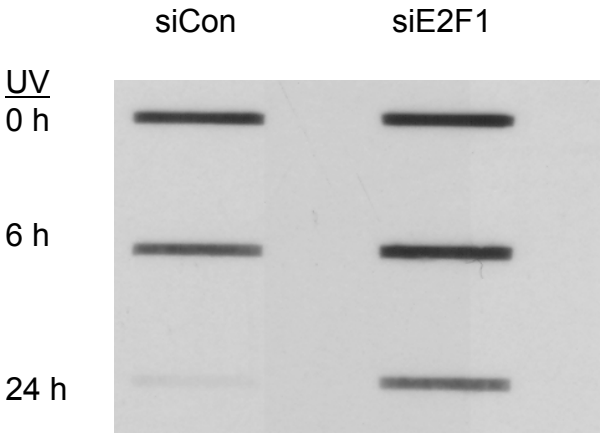
MTT Viability Assay



Knockdown of E2F1 Reduces DNA Repair Efficiency of UV Photoproducts

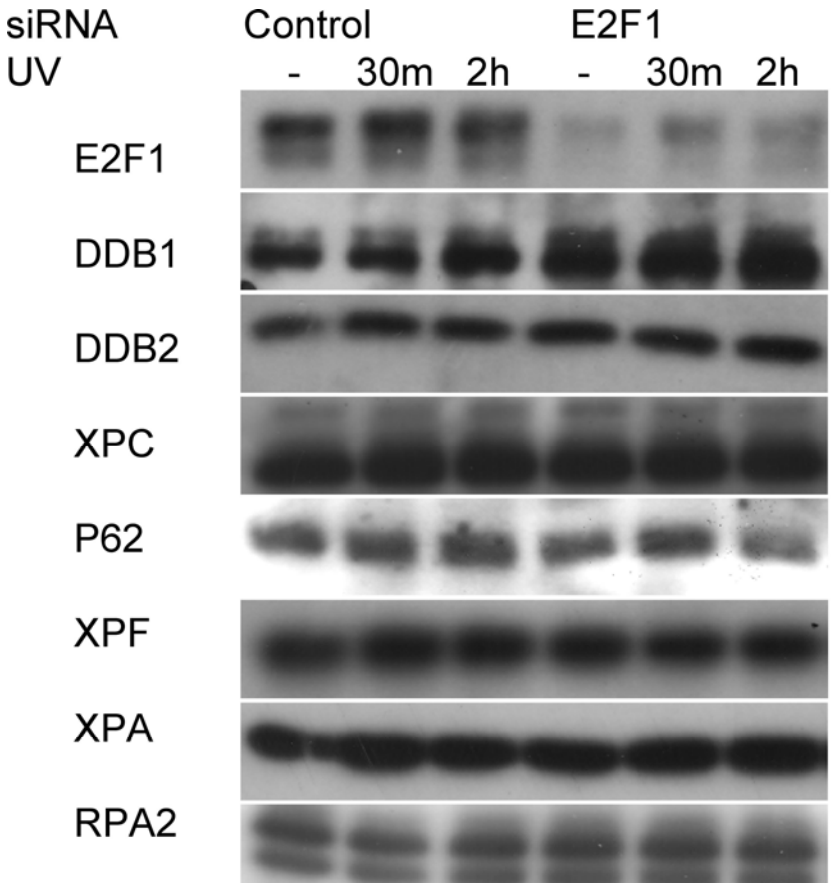


Anti-6-4 PP

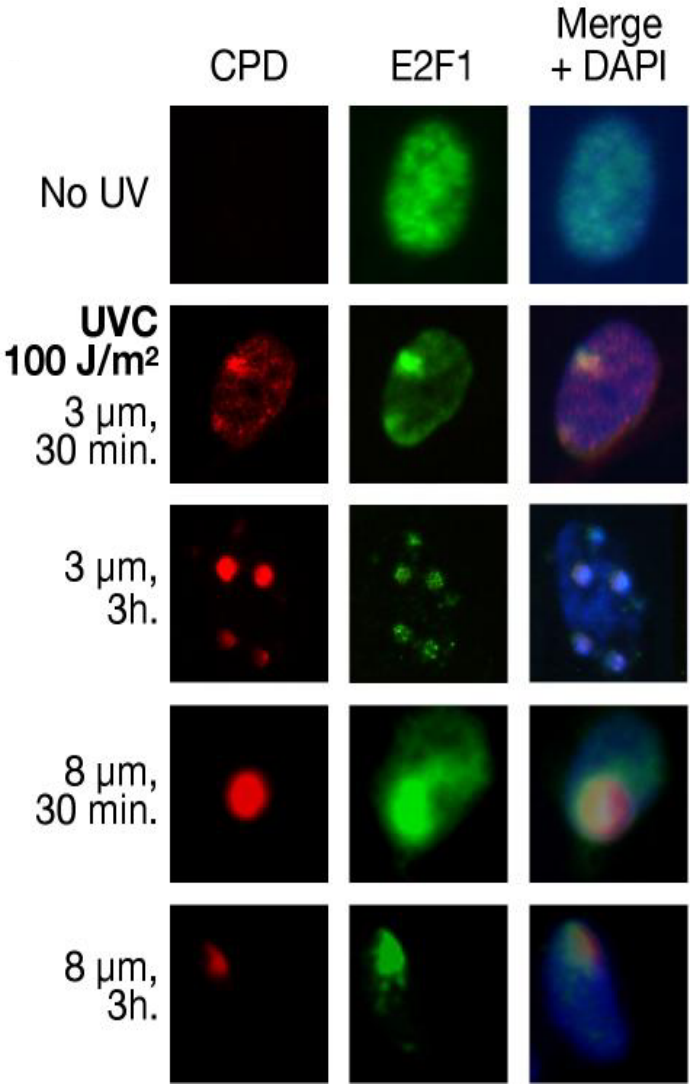


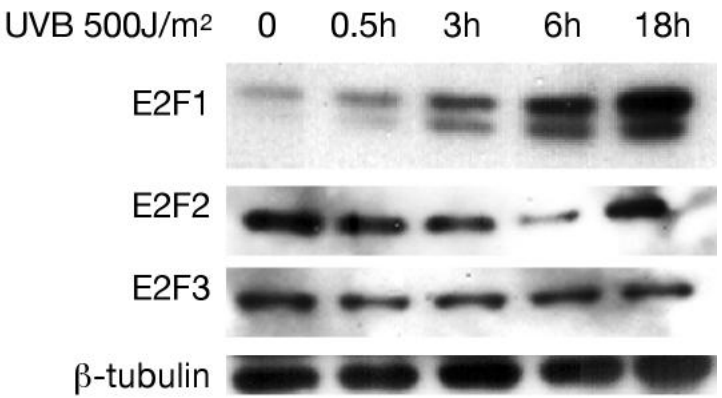
Anti-CPD

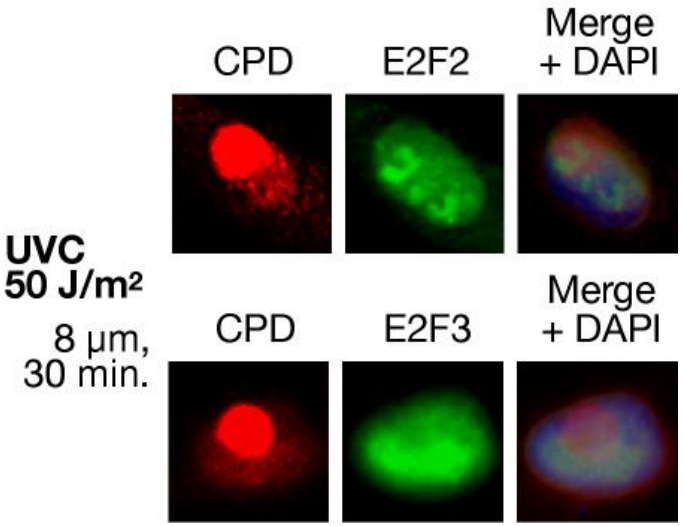
E2F1 Deficiency Does Not Alter the Expression of NER Factors



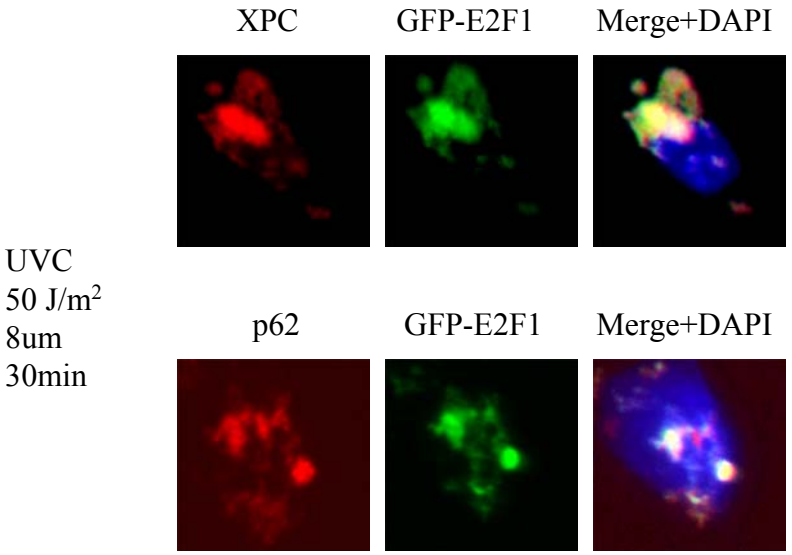
E2F1 Accumulates at Sites of UV-induced Damage

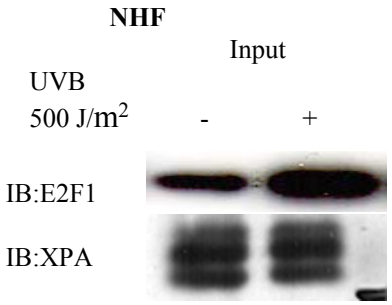


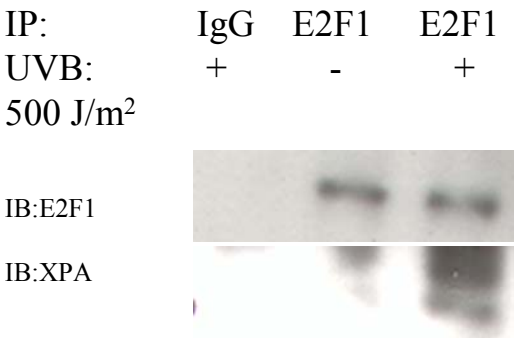




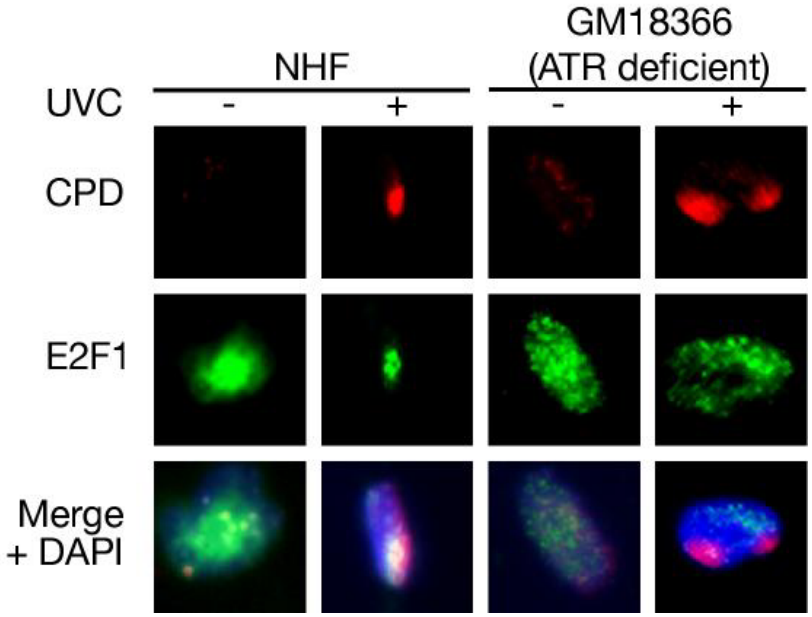
E2F1 Co-localizes with XPC and p62 (TFIIH) and Associates with XPA in Response to UV Irradiation

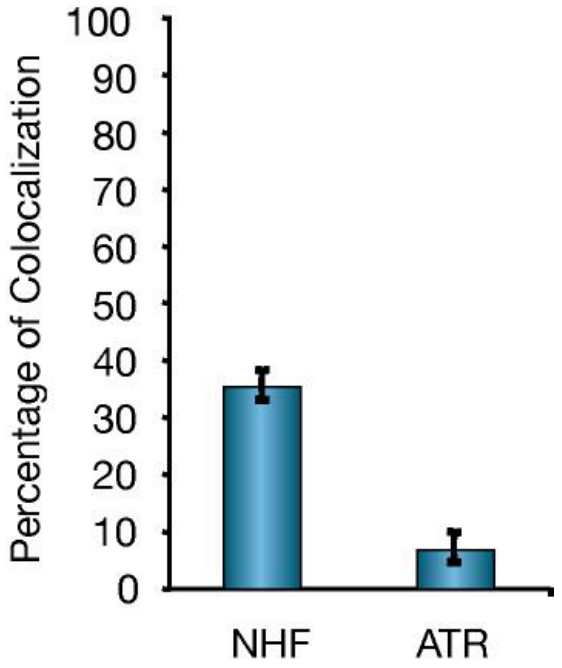




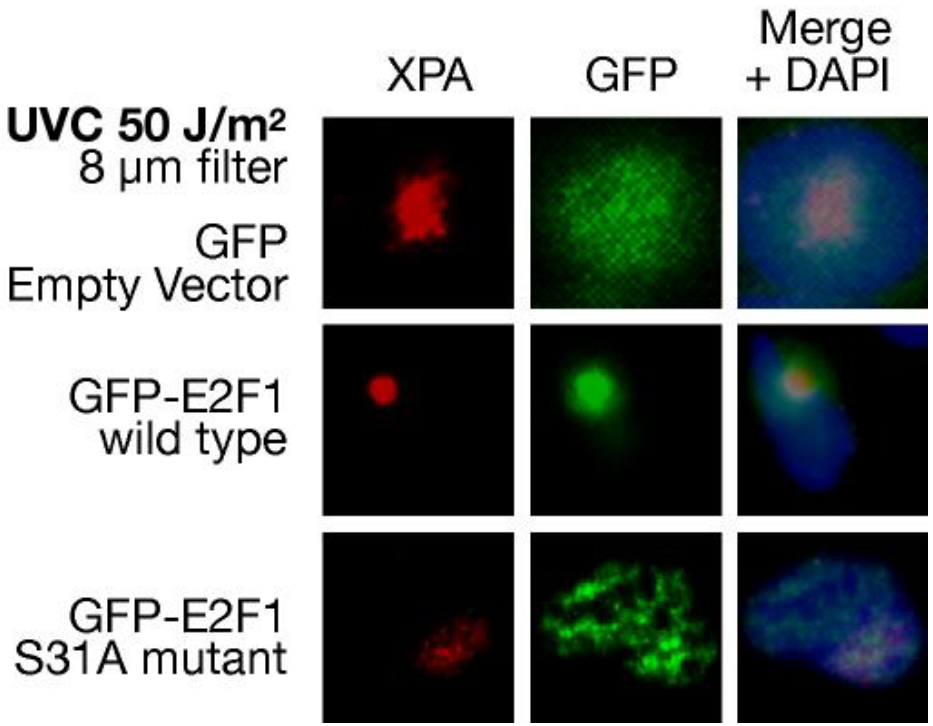


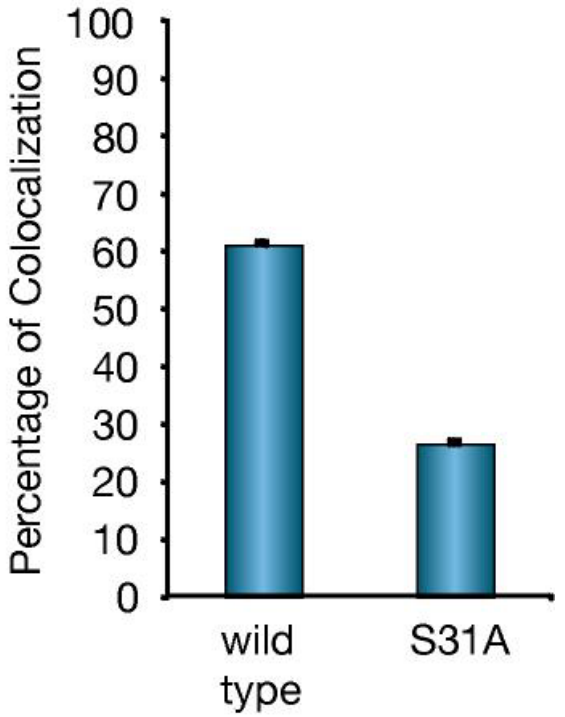
E2F1 Localization to Sites of UV Damage Requires ATR



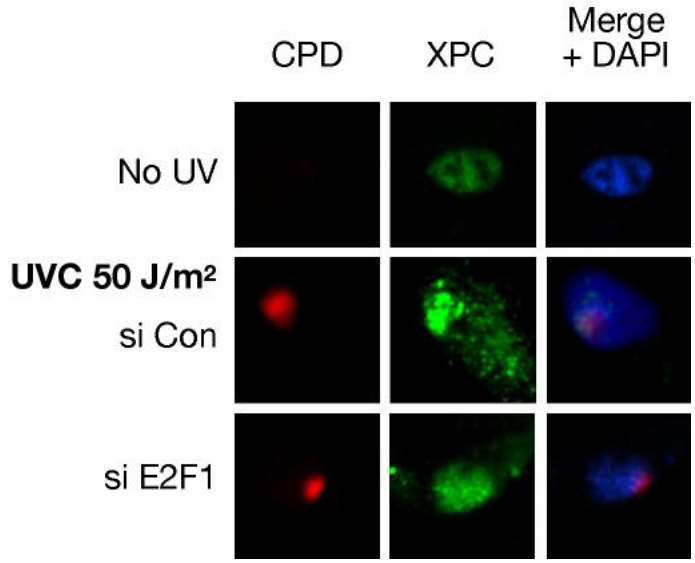


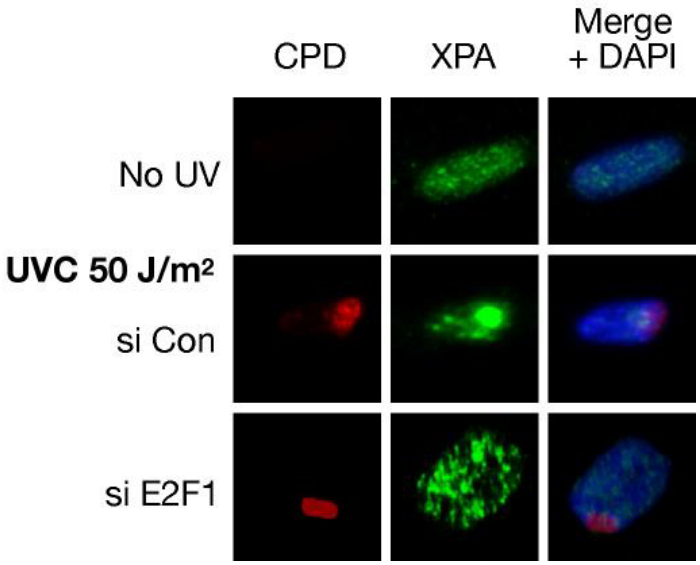
E2F1 Serine 31 is Required for Localization to Sites of UV Damage

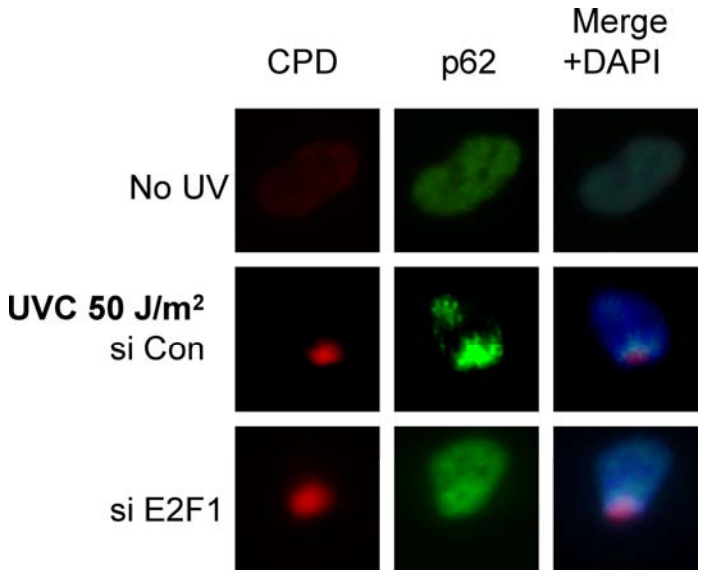


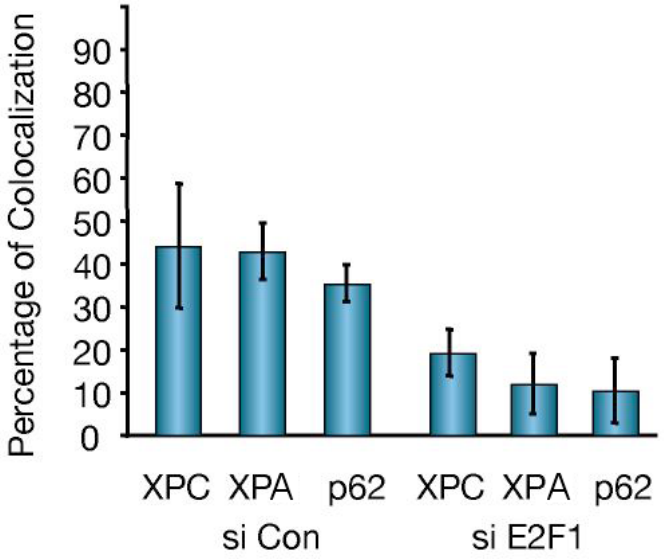


Knockdown of E2F1 Impairs Recruitment of NER Factors to Sites of UV Damage

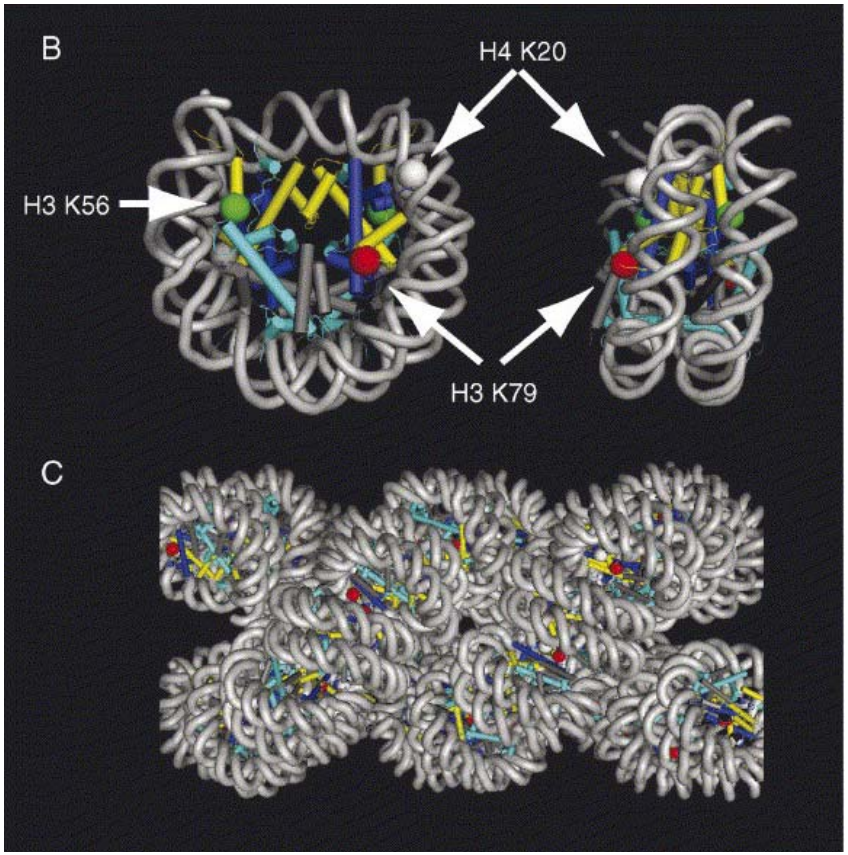








Histone Acetylation and Chromatin Relaxation in Response to UV-induced DNA Damage

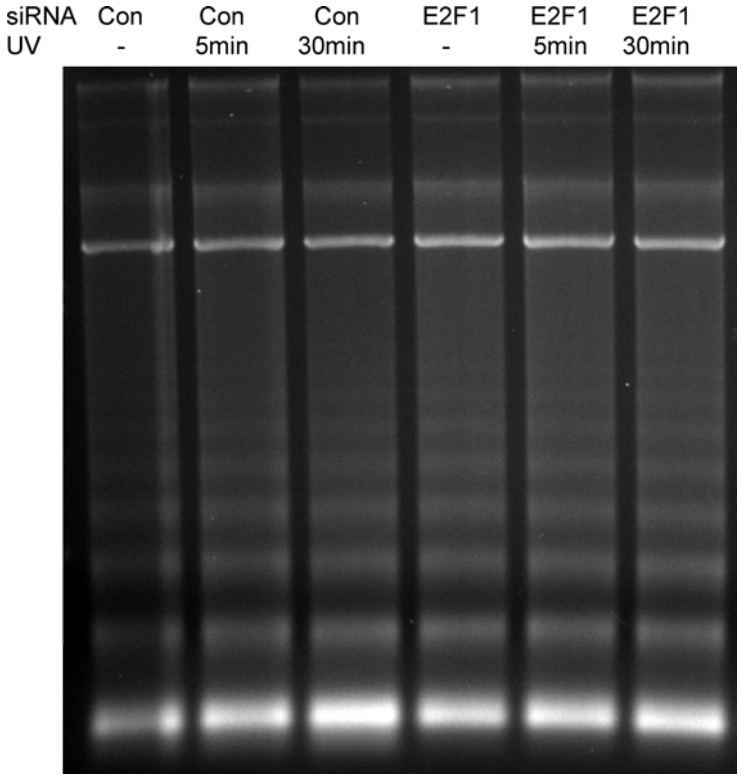


- Experiments by the Frieberg (1991) and Sancar (2000) groups demonstrated that nucleosomes can inhibit NER.

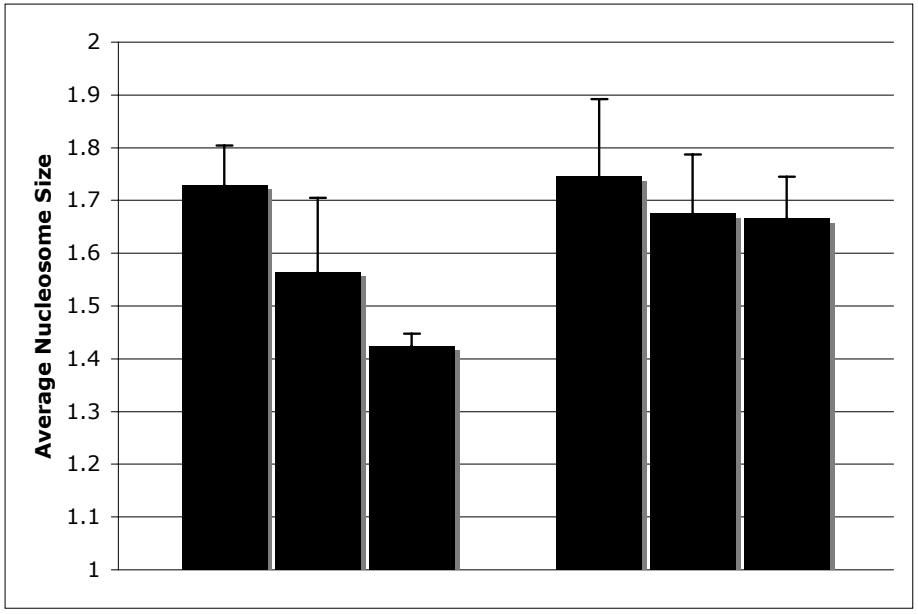
- Smerdon and coworkers originally demonstrated that UV irradiation increased histone acetylation, which stimulated DNA repair (1982-1986).

- Other groups have demonstrated that p53, ING1/2, and p300 participate in a pathway that promotes repair by inducing histone H4 acetylation, chromatin relaxation, and increased accessibility to NER factors.

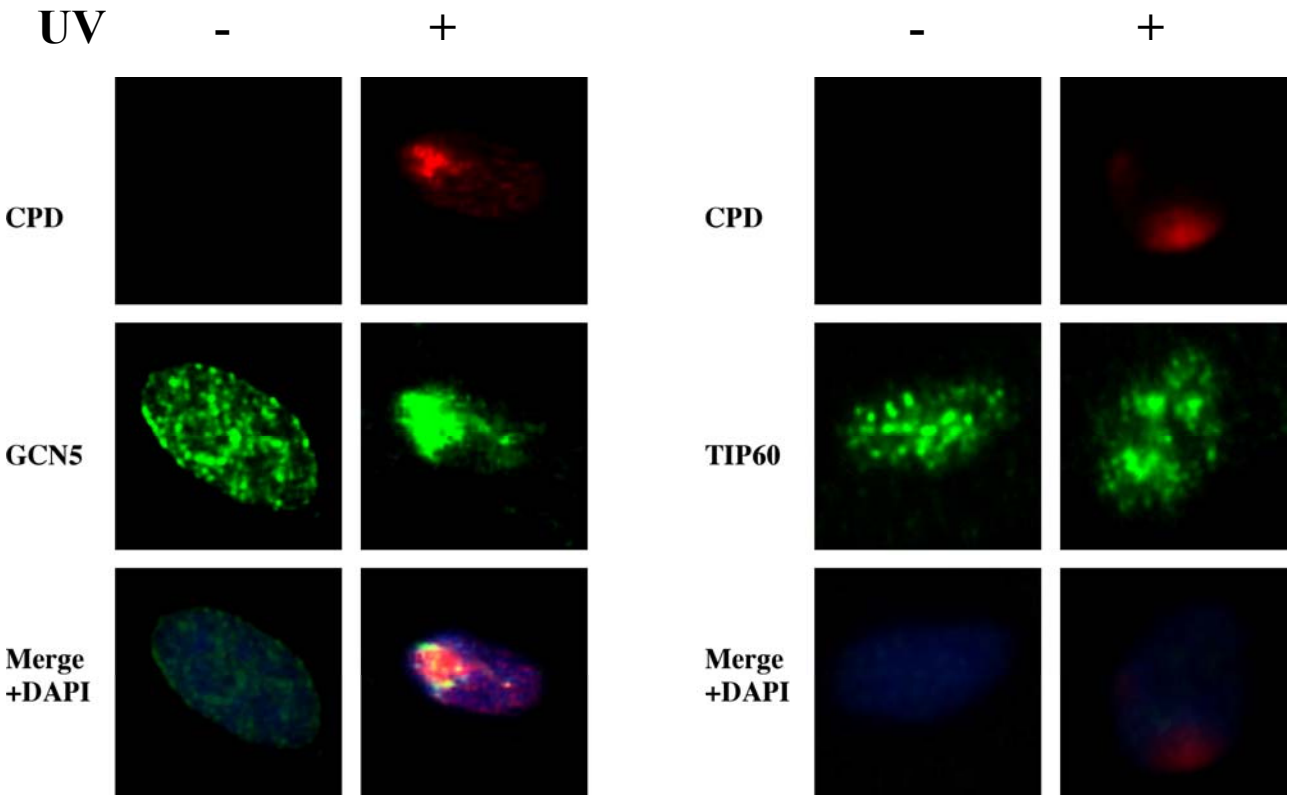
Chromatin Relaxation in Response to UV Is Impaired by E2F1 Deficiency



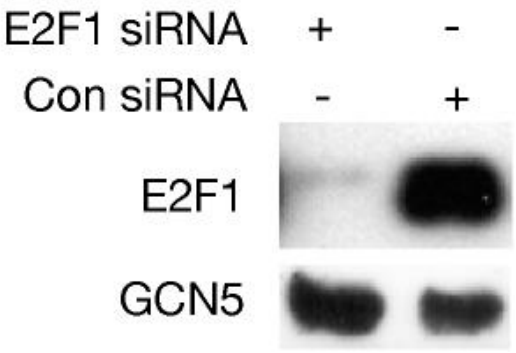
Micrococcal Nuclease Assay

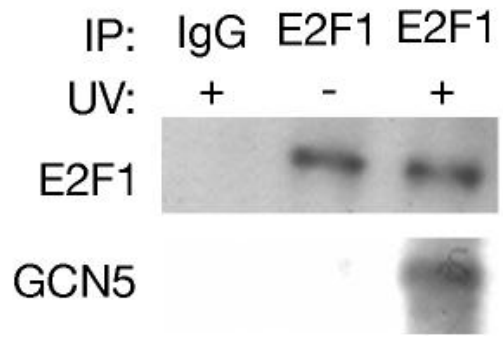


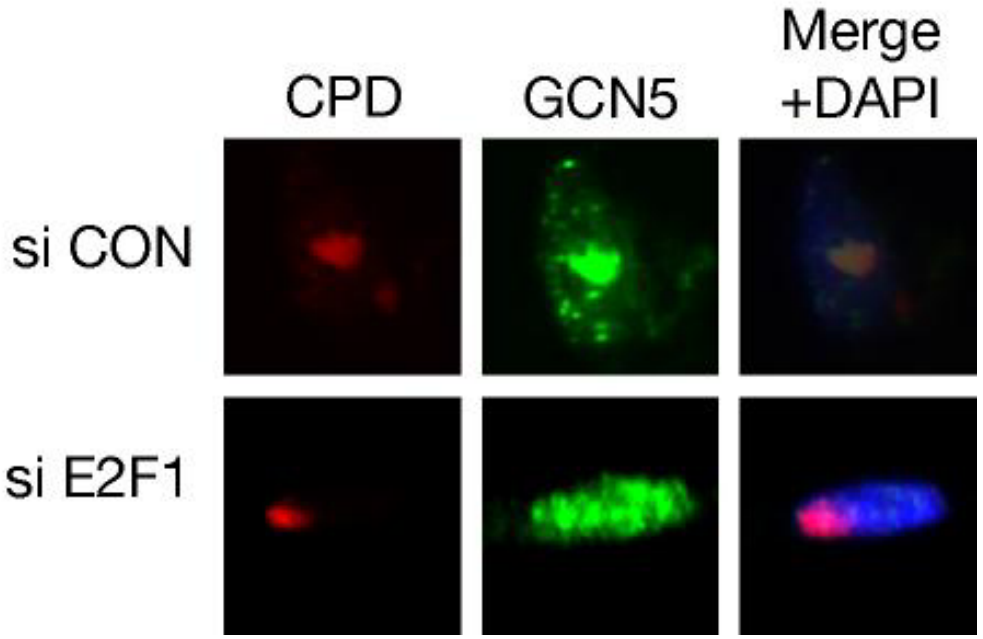
The GCN5 Histone Acetyltransferase (HAT) Accumulates at Sites of UV damage



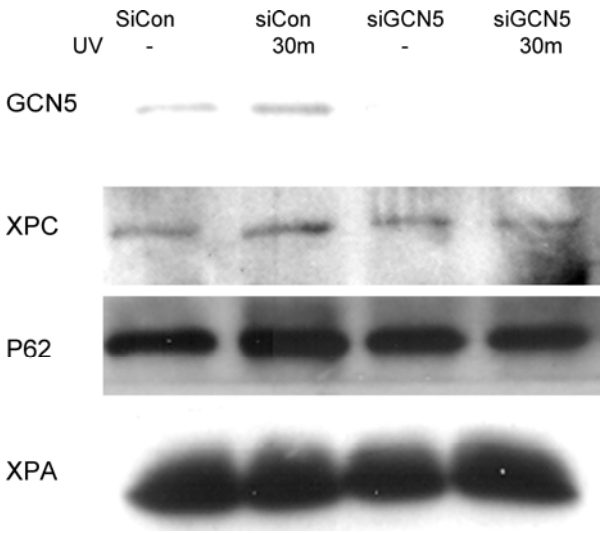
E2F1 Interacts with GCN5 Following UV Irradiation and Recruits GCN5 to Sites of Damage

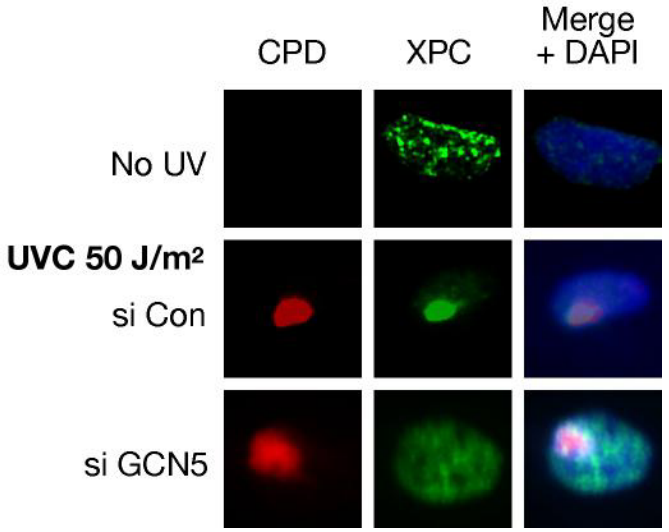


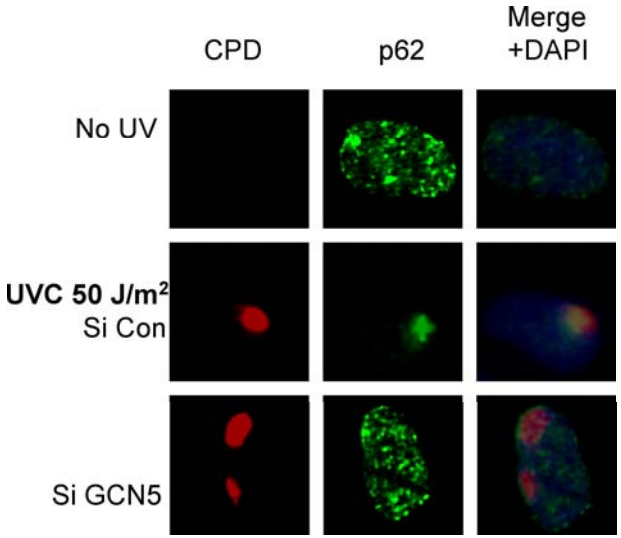


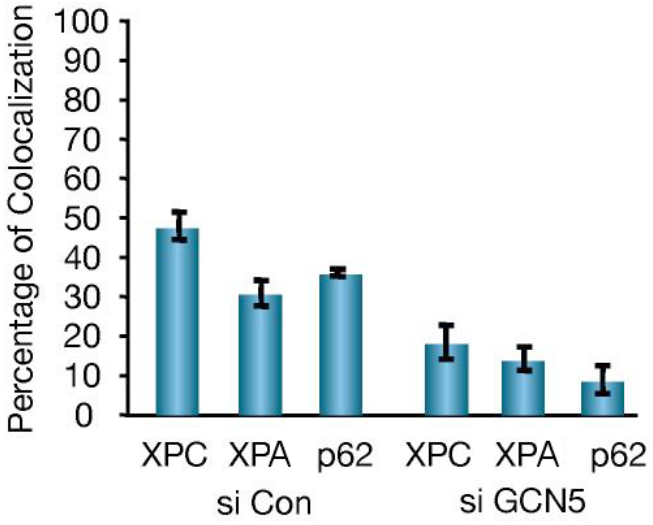


Knockdown of GCN5 Impairs Recruitment of NER factors to Sites of UV Damage

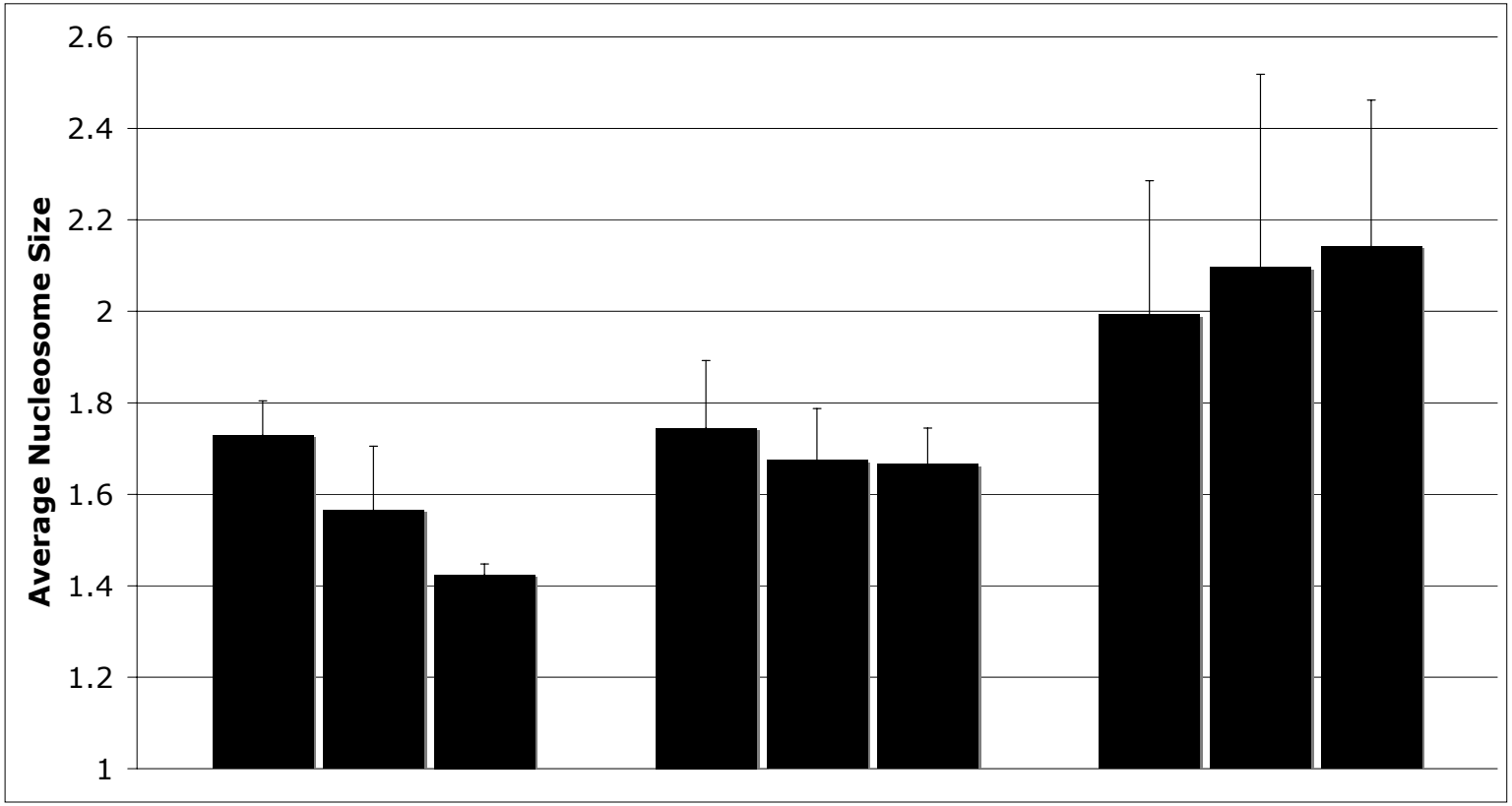




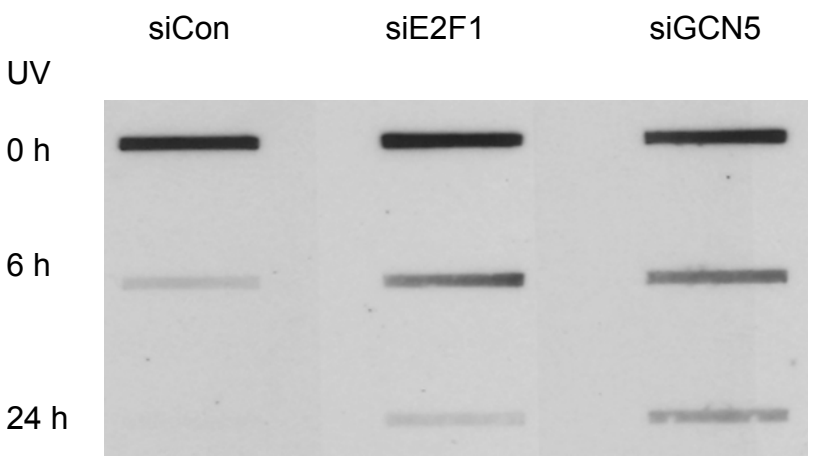




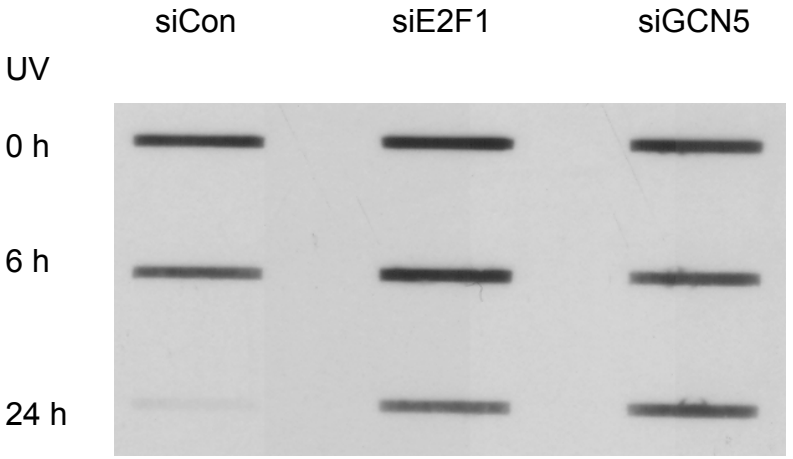
Knockdown of GCN5 Prevents Chromatin Relaxation in Response to UV



Knockdown of E2F1 or GCN5 Reduces DNA Repair Efficiency

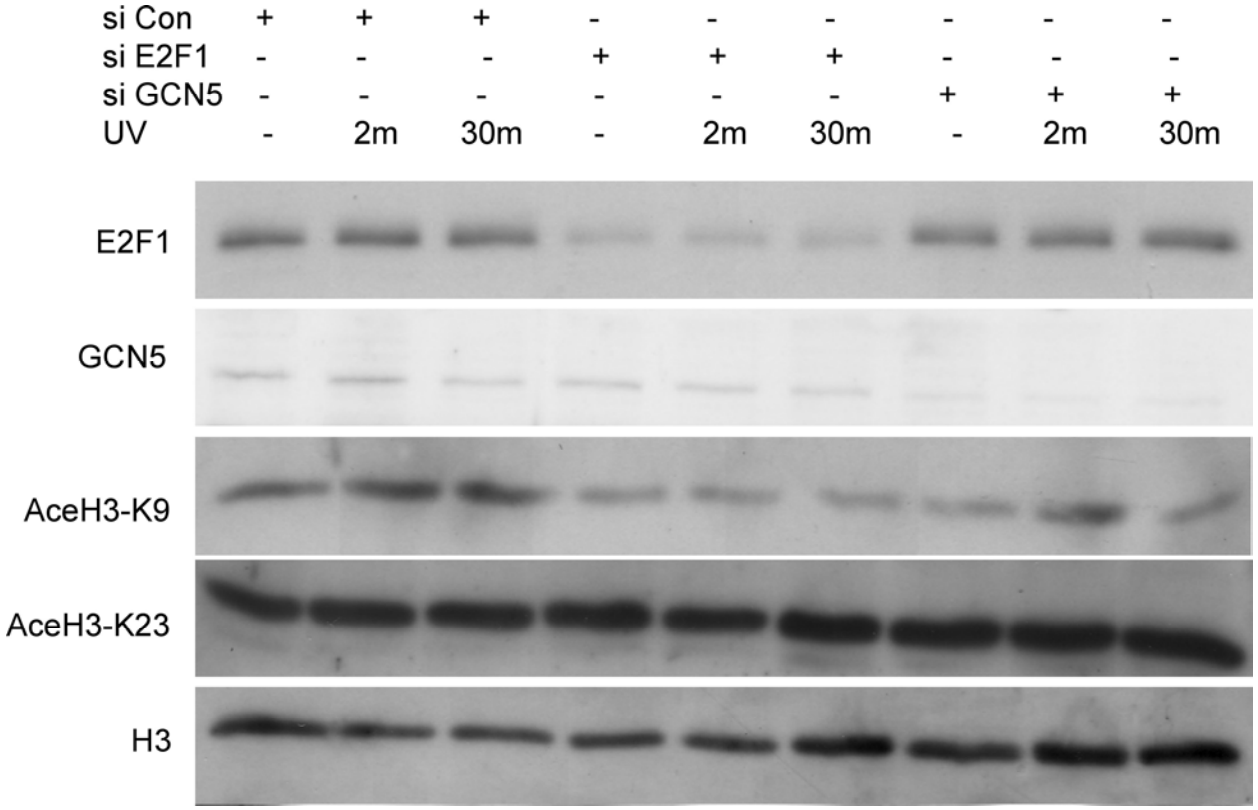


Anti-6-4 PP

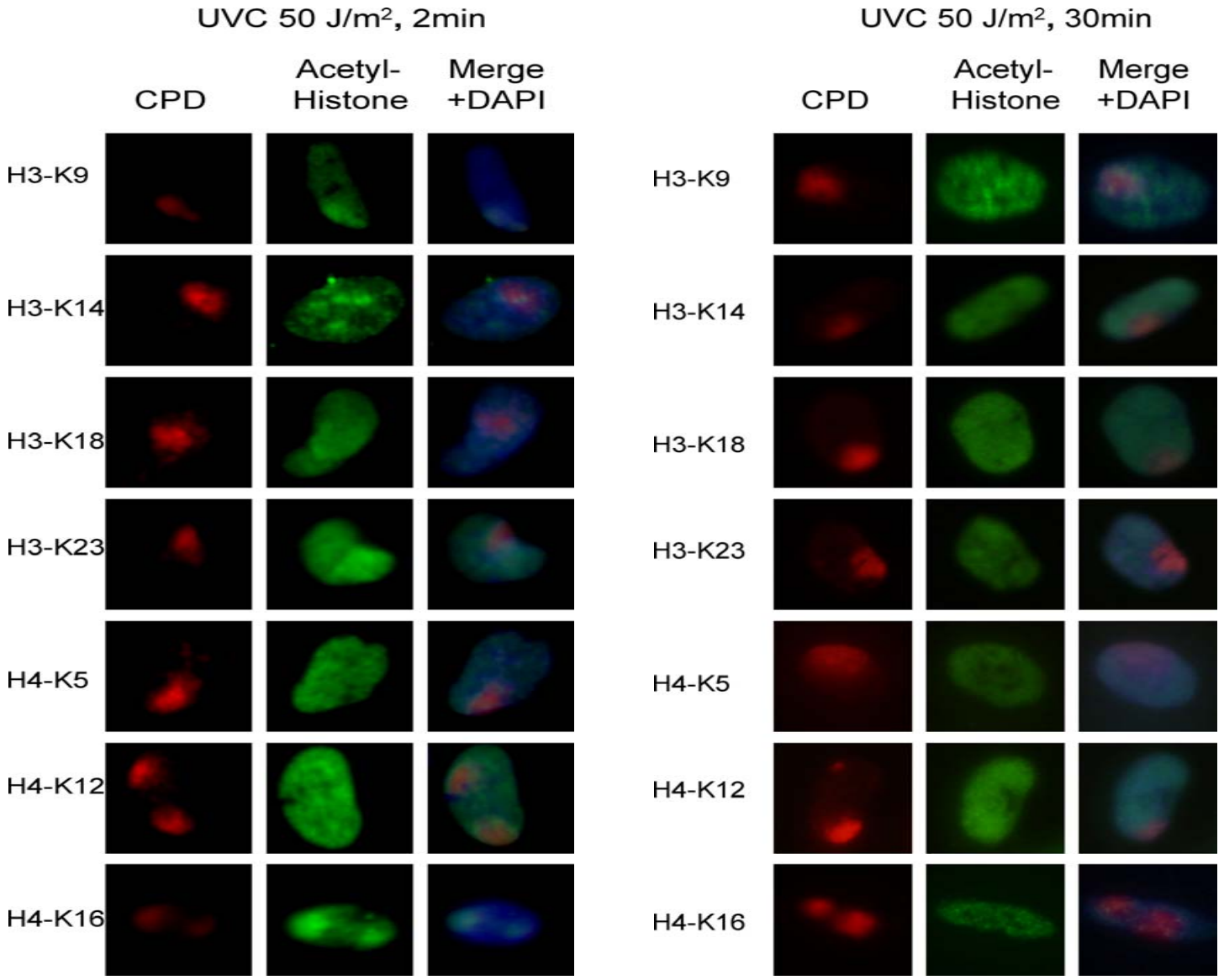


Anti-CPD

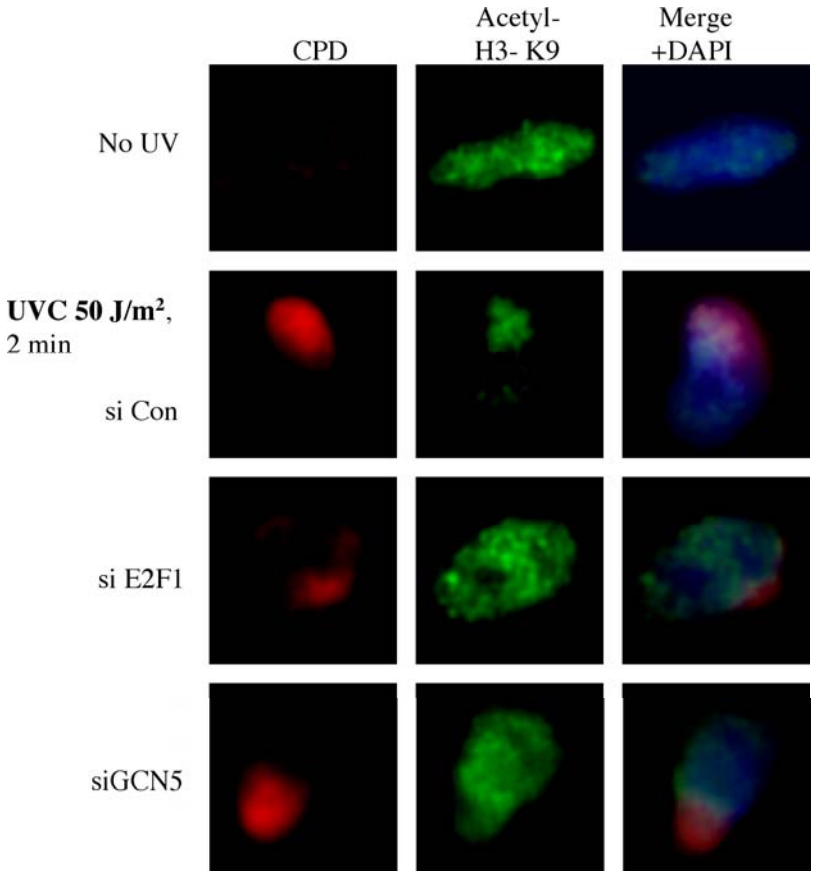
Increased H3 K9 Acetylation in Response to UV Is Impaired by E2F1 or GCN5 Deficiency

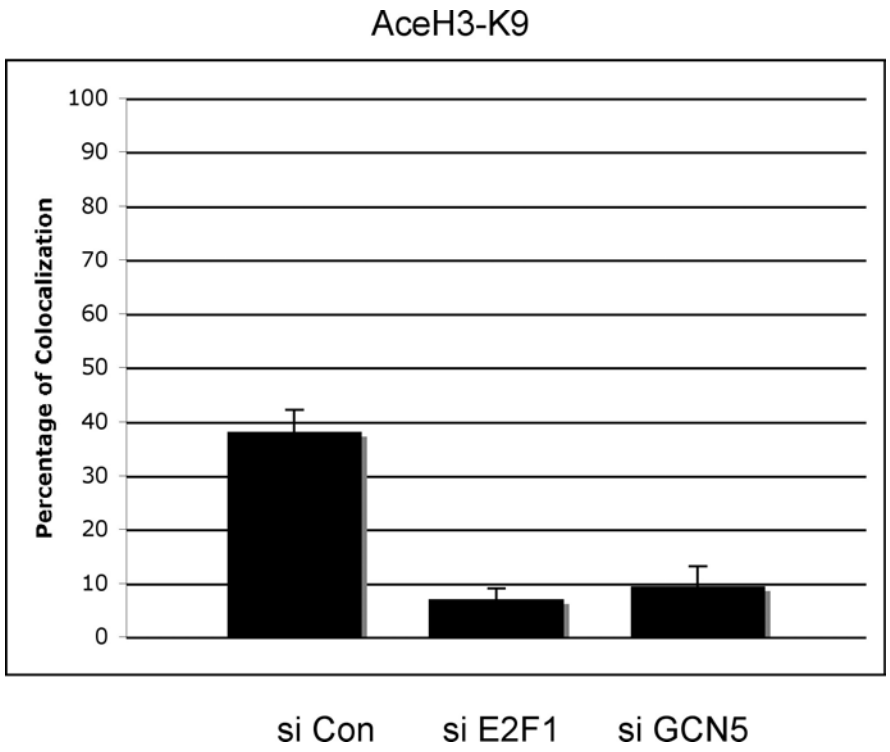


Rapid Accumulation of Acetylated H3 K9 and H4 K16 at Sites of UV damage

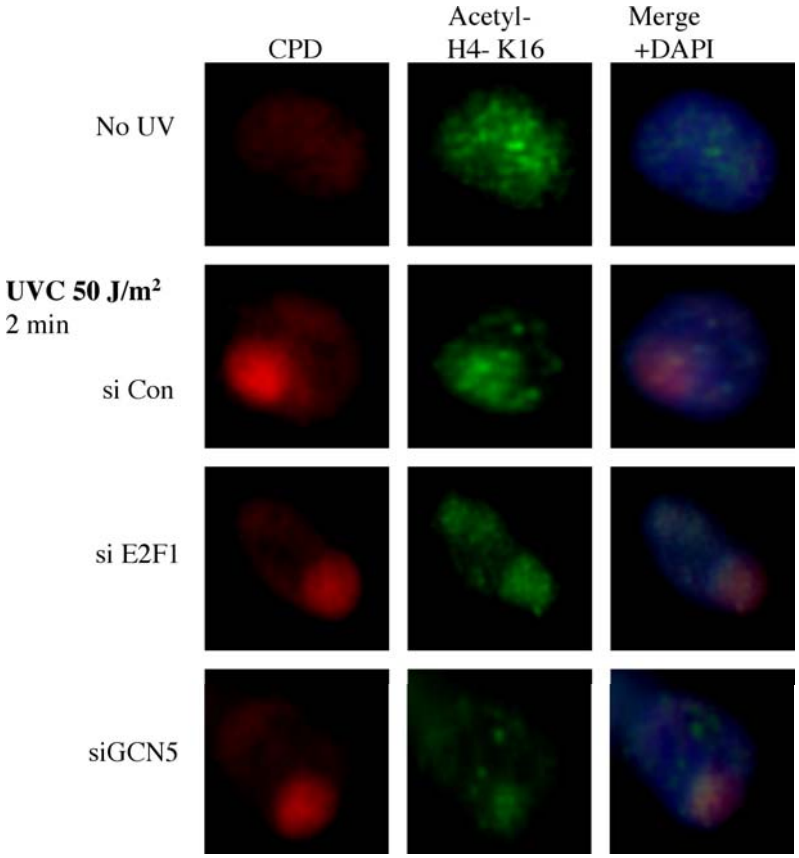


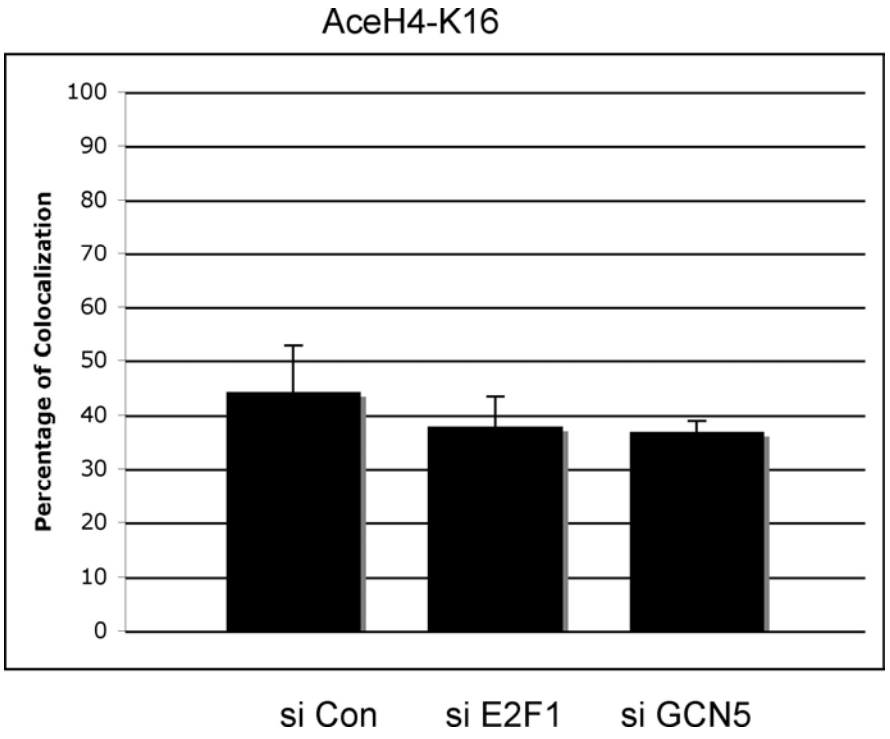
Knockdown of E2F1 or GCN5 Impairs H3 K9 Acetylation at Sites of UV Damage



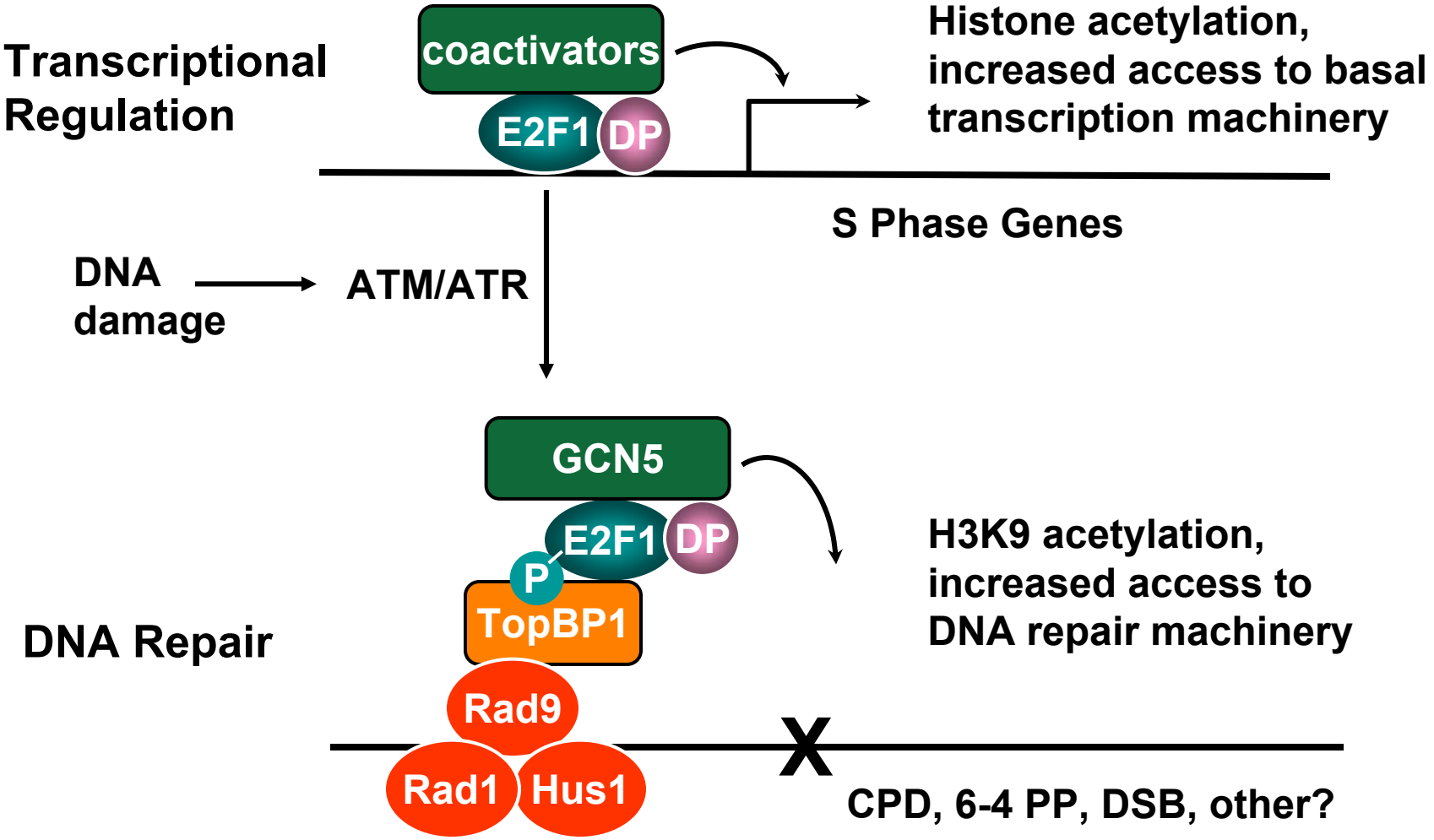


E2F1 and GCN5 Are Not Required for H4 K16 Acetylation at Sites of UV Damage

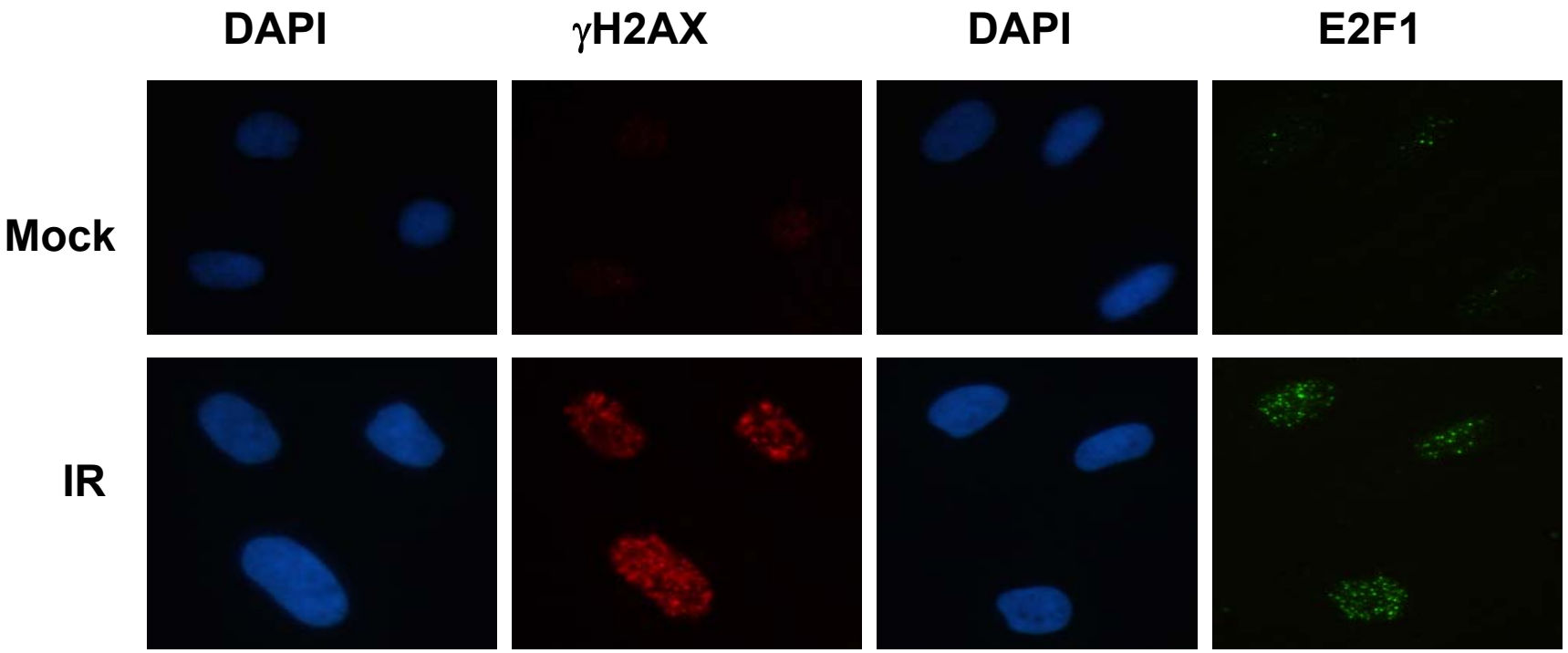




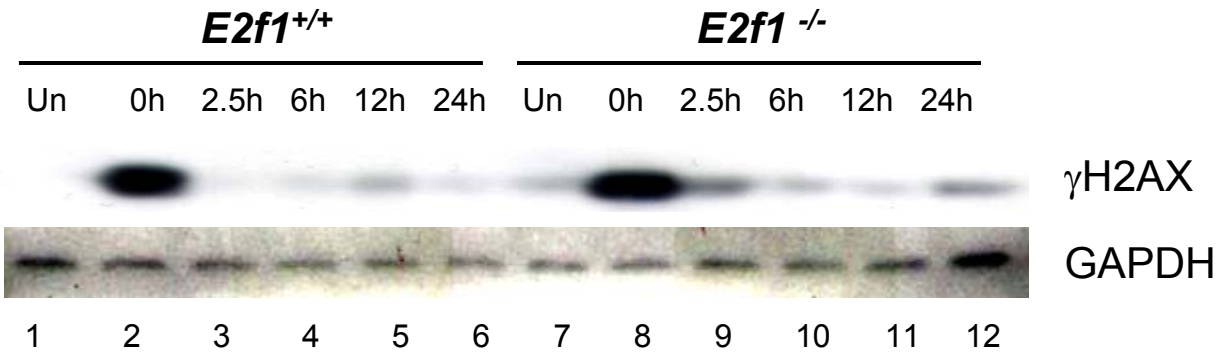
The DNA Damage Response Converts E2F1 Into an Accessibility Factor for DNA Repair



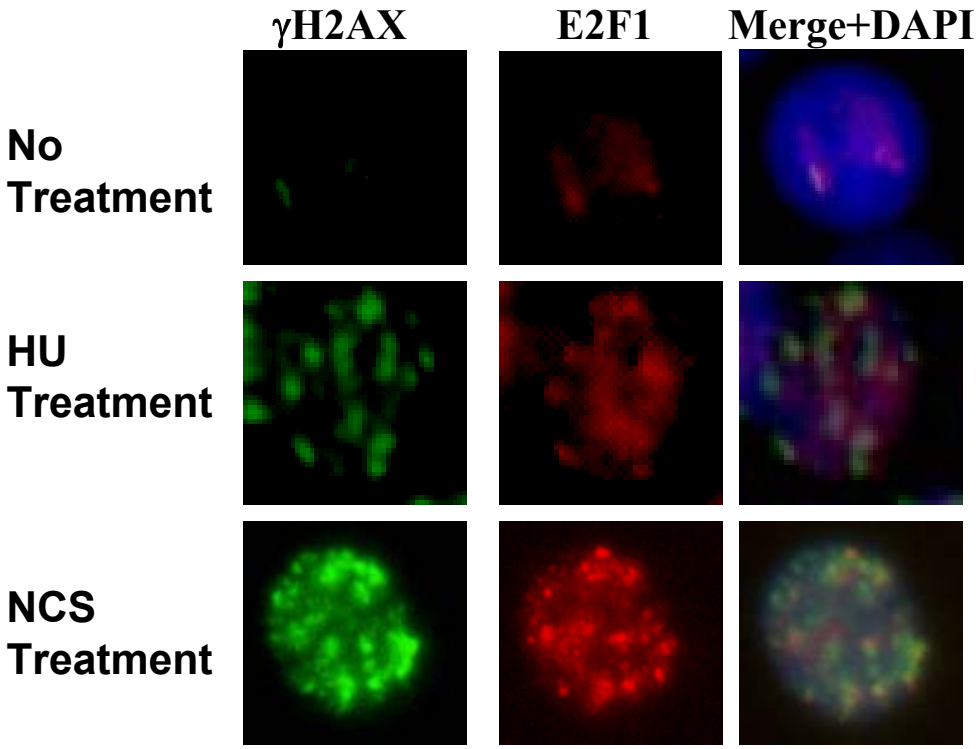
E2F1 Localizes to Nuclear Foci in Response to Ionizing Radiation



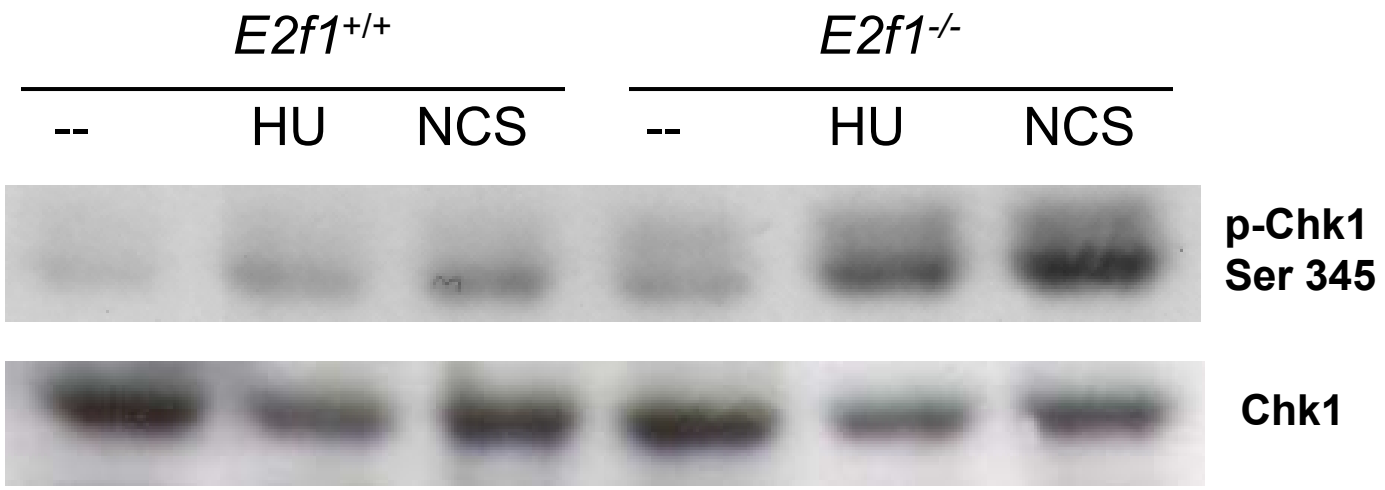
Inactivation of E2F1 Affects the Kinetics of H2AX Phosphorylation Following IR Exposure



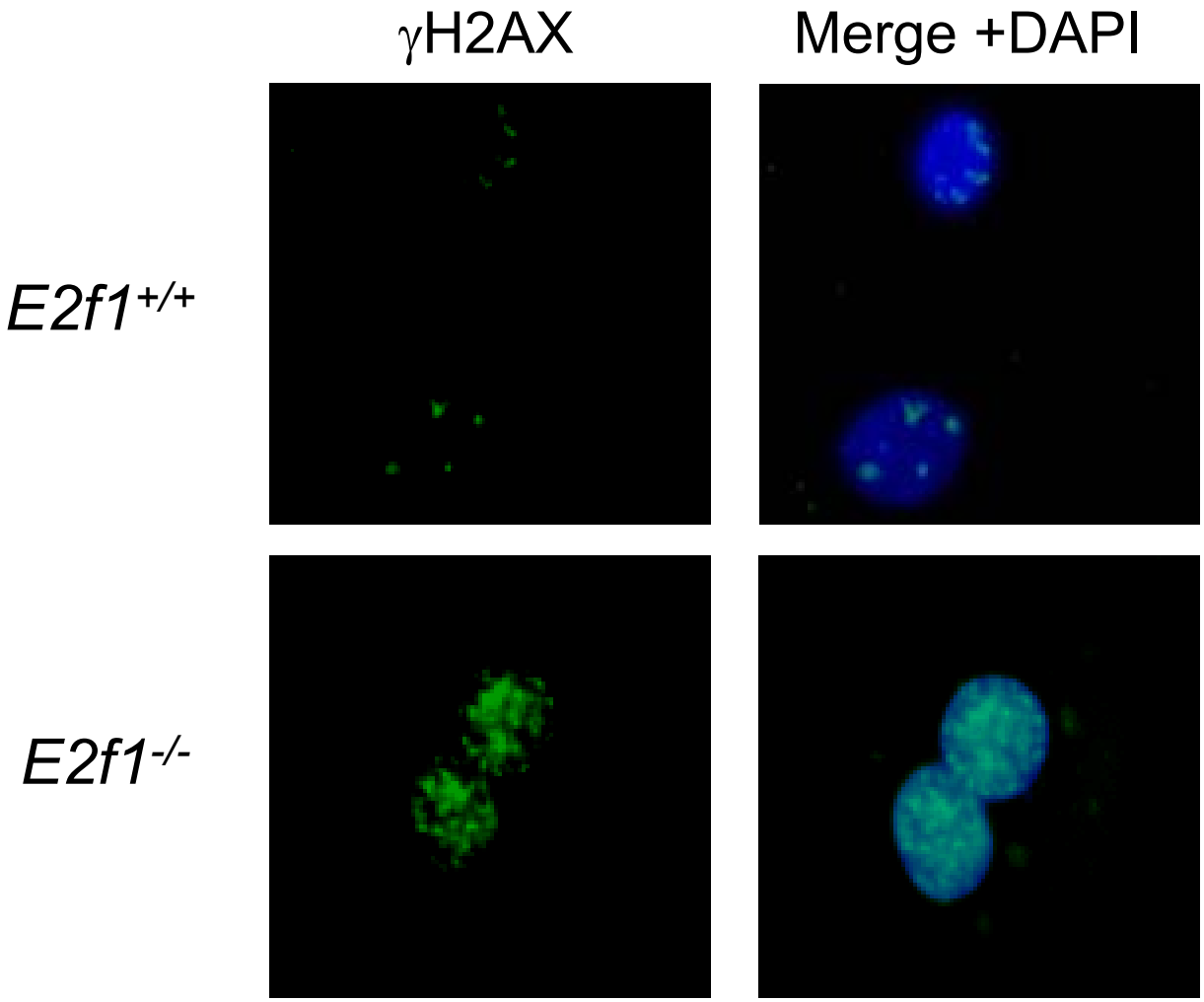
E2F1 Foci Formation in Cells Treated with Hydroxyurea (HU) and Neocarzinostatin (NCS)



Increased Chk1 Activation in the Absence of E2F1



Accumulation of DNA damage in Untreated Primary *E2f1*$^{-/-}$ Cells



Cytogenetic Analysis of Primary Keratinocytes Indicates Genomic Instability in the Absence of E2F1

Genotype	% aberrant met.	% with breaks	% with fragments	% with fusions
Wild type	13.0	10.3	0	4.6
E2f1$^{-/-}$	31.5	18.6	4.6	17.4

Future Directions

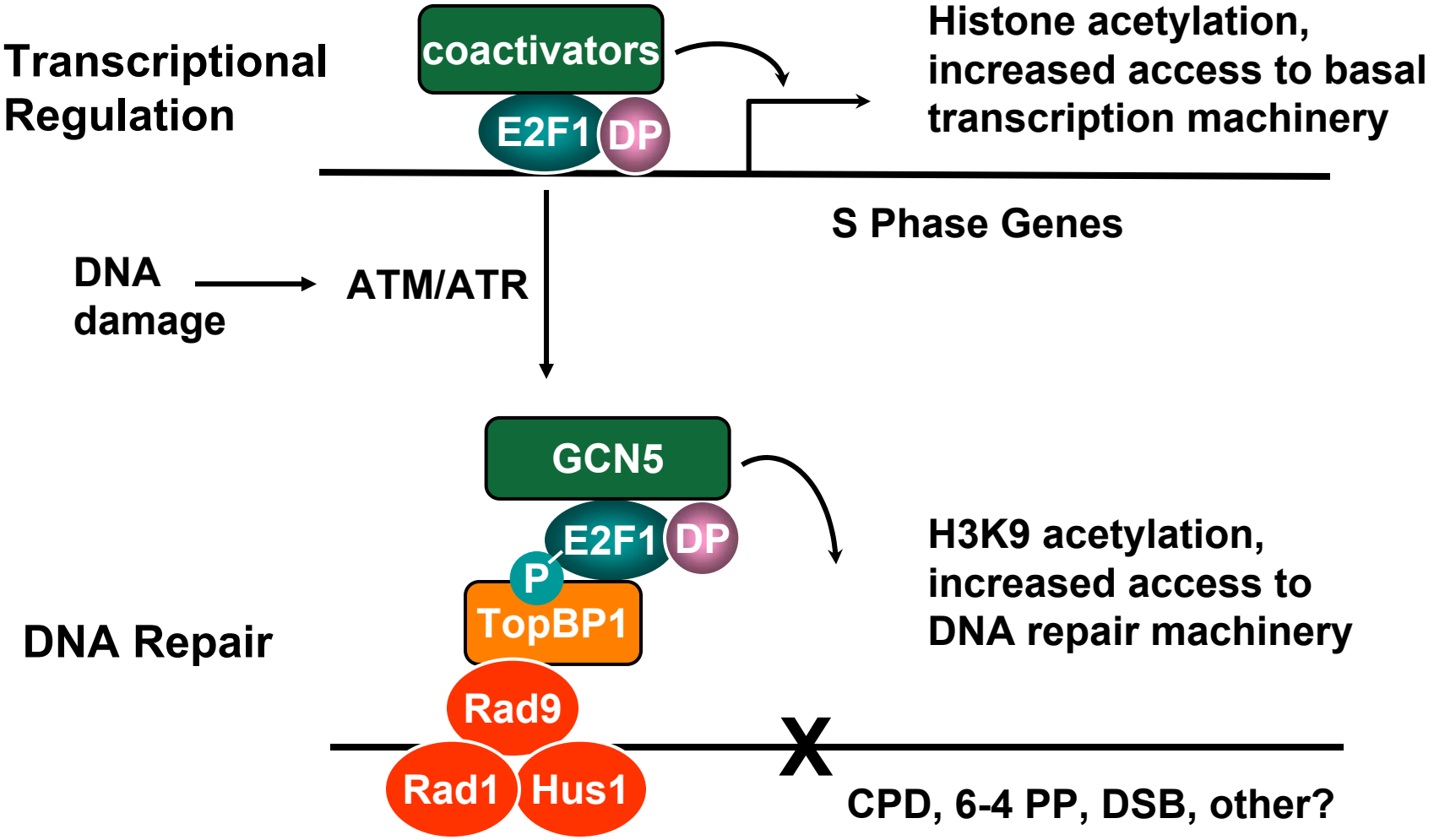
- Determine how E2F1 is recruited to sites of DNA damage.

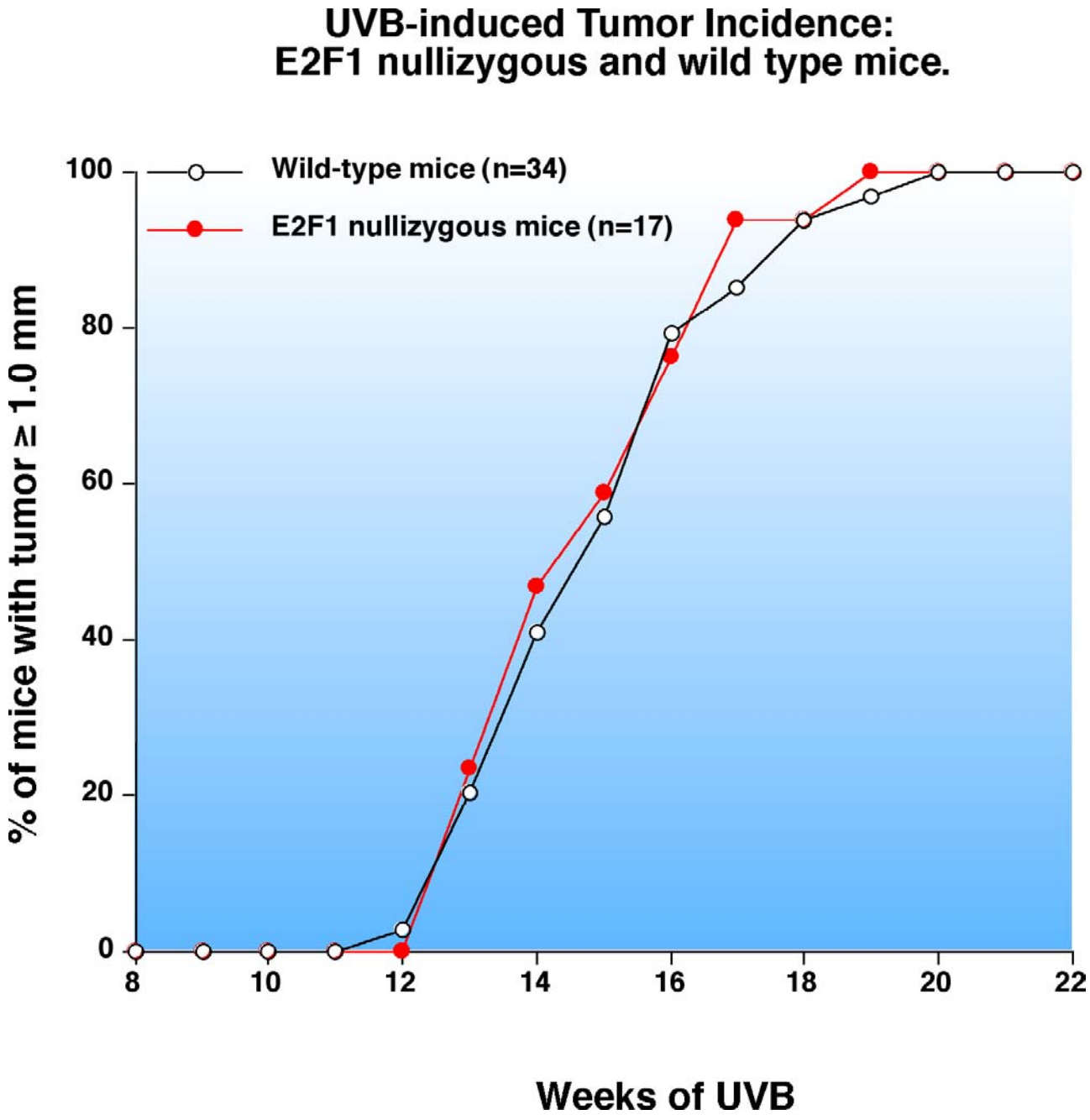
- Determine how E2F1 recruits GCN5 to sites of DNA damage.

- Determine if and how E2F1 participates in the repair of double-strand breaks and perhaps other types of DNA damage.

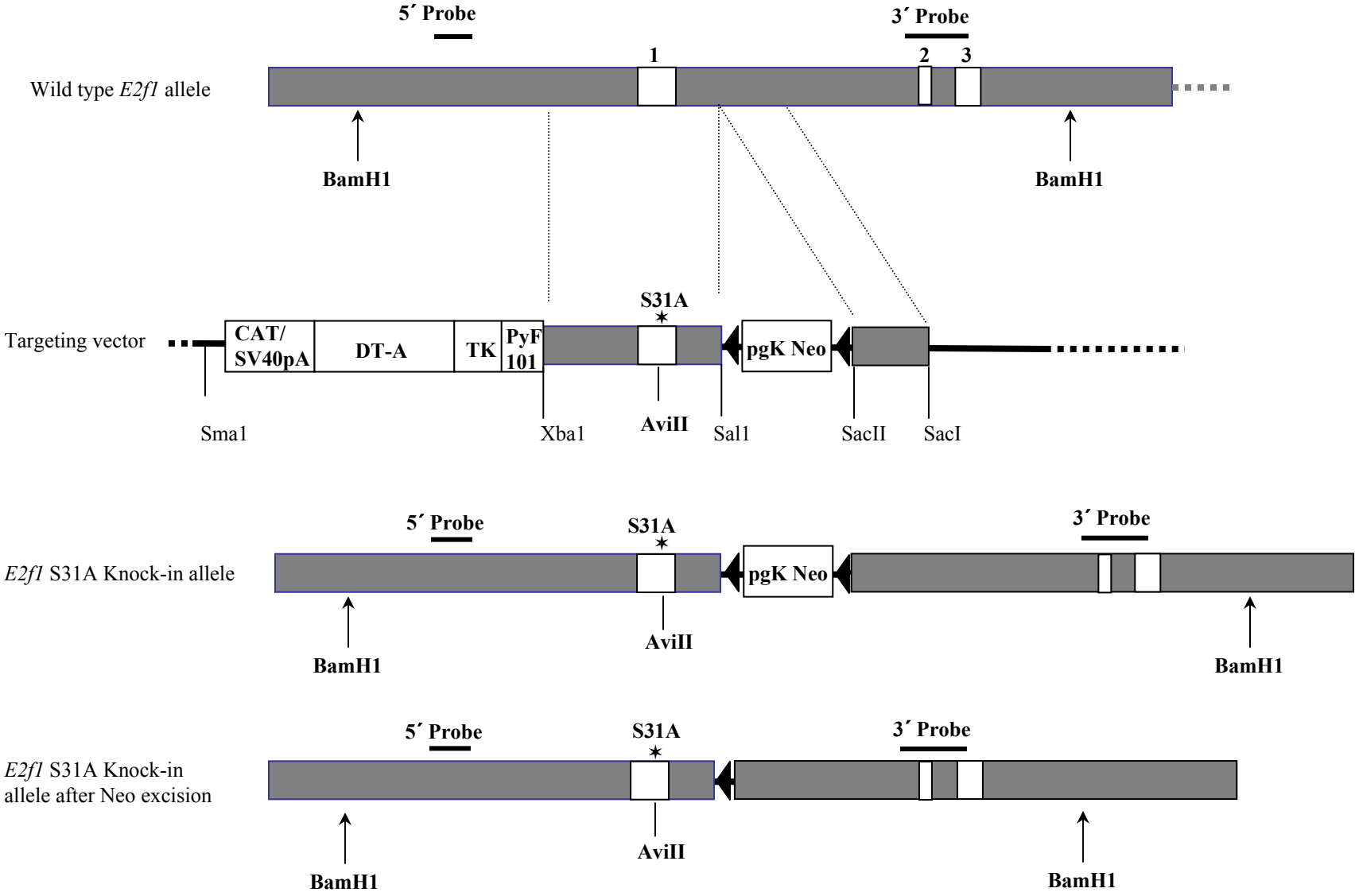
- Determine if E2F1 associates with other chromatin modifiers and mediates other histone modifications in response to DNA damage.

The DNA Damage Response Converts E2F1 Into an Accessibility Factor for DNA Repair

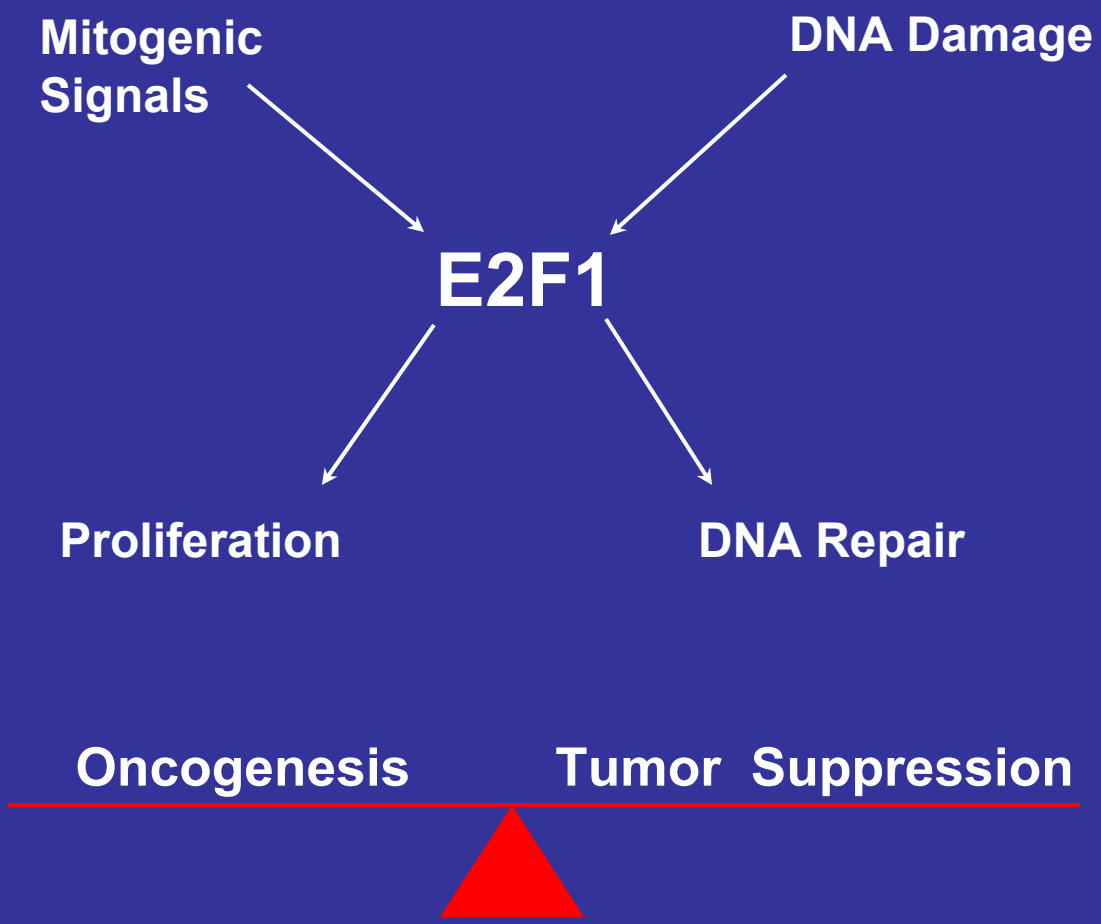



UVB-induced Tumor Incidence:
E2F1 nullizygous and wild type mice.
Wild-type mice (n=34)
E2F1 nullizygous mice (n=17)
% of mice with tumor ≥ 1.0 mm
100
80
60
40
20
0
8
10
12
14
16
18
20
22
Weeks of UVB

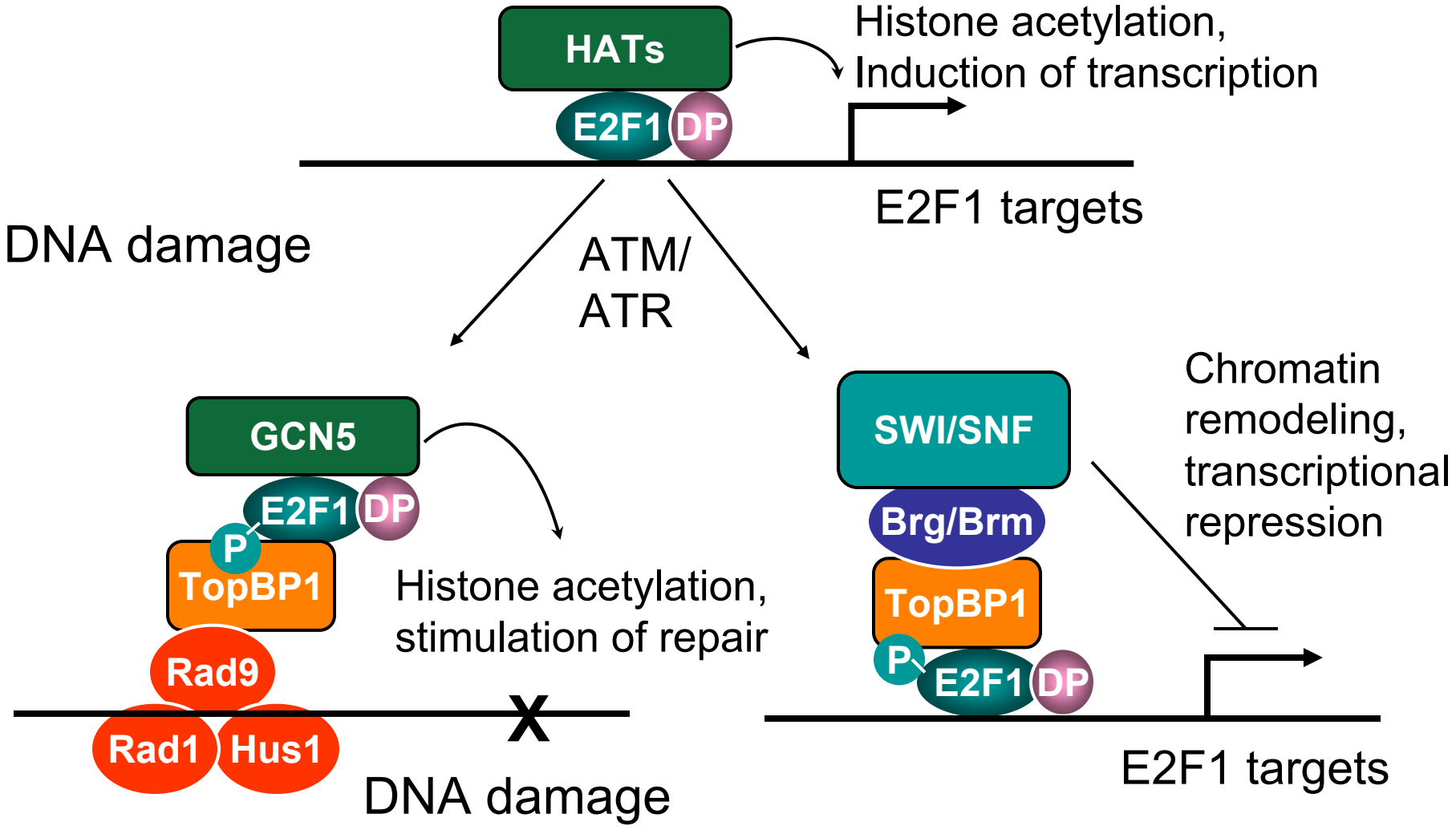
E2F1 S31A Knock in Scheme



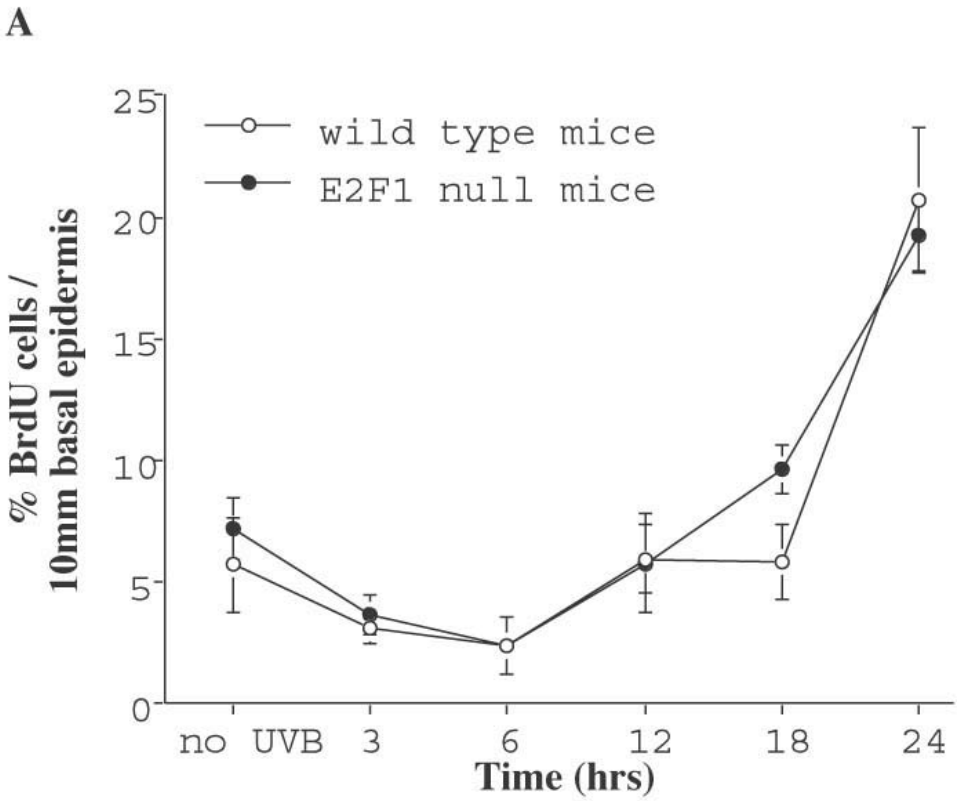
E2F1 Can Behave as Both an Oncogene and Tumor Suppressor Gene in Mouse Models



DNA damage converts E2F1 into a transcriptional repressor as well as a DNA repair factor



Differences in Photoproduct Removal Are Not Due to Differences in DNA Synthesis



E2f1; *p53* Double Knockouts are Hypersensitive to UV-induced Apoptosis