**DNA-PK suppresses a p53 independent apoptotic response to DNA damage**

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**ABSTRACT**

p53 is required for DNA damage induced apoptosis, which is central to its function as a tumor suppressor. Here we show the apoptotic defect of p53 deficient cells is nearly completely rescued by inactivation of any of the three subunits of the DNA repair holoenzyme DNA-PK. Intestinal crypt cells from *p53* nullizygous mice were resistant to radiation induced apoptosis, while apoptosis in *DNAPK$_{cs}$/p53*, *Ku80/p53*, and *Ku70/p53* double null mice was quantitatively equivalent to that seen in wild type mice. This p53 independent apoptotic response was specific to the loss of DNA-PK, as it was not seen in *Ligase IV/p53* or *ATM/p53* double null mice. Further, it was associated with increased phospho-Chk2, and cleaved caspases 3 and 9, the latter indicating engagement of the intrinsic apoptotic pathway. This demonstrates there are two separate, but equally effective, apoptotic responses to DNA damage, one is p53 dependent and the other, engaged in the absence of DNA-PK, does not require p53.

## INTRODUCTION

The tumor suppressor p53 plays a central role in the cellular response to DNA damage. After DNA damage, p53 is rapidly phosphorylated by the Atm and Atr protein kinases, leading to its stabilization, induction of its transcriptional targets, and cell cycle arrest or apoptosis (Vousden and Lu, 2002;Shiloh, 2003). p53 deficient cells fail to undergo G1 cell cycle arrest or apoptosis in response to DNA damage, underlying the critical role of p53 in these responses. The apoptotic resistance of p53 deficient cells could have clinical implications, as p53 is frequently mutated in human tumors and p53 deficient tumor models have reduced sensitivity to radio- or chemo-therapy (Johnstone et al., 2002). However, in some settings, p53 deficient cells can undergo apoptosis (Strasser *et al.*, 1994;Merritt *et al.*, 1997;Roos and Kaina, 2006) suggesting there are alternative cell death pathways. Understanding p53 independent cell death pathways and harnessing them to enhance the sensitivity of tumor cells to chemo or radiotherapy could have therapeutic benefit (Brown and Attardi, 2005).

The DNA double strand break (dsb) is the major DNA lesion that activates p53-dependent apoptosis (Nelson and Kastan, 1994). In addition to triggering programmed cell death, dsbs activate repair machinery to rejoin the breaks through either homologous recombination (HR) or non homologous end joining (NHEJ) (Shrivastav et al., 2008). During NHEJ, two subunits, Ku70 and Ku80, form a heterodimer that binds to the broken DNA ends, which then recruits the catalytic subunit of DNA-PK (DNA-$PK_{cs}$) (Smith and Jackson, 1999). DNA breaks are synapsed together by DNA-$PK_{cs}$ (DeFazio *et al.*, 2002;Spagnolo *et al.*, 2006) and processed to remove overhanging 3' or 5' ends, so they can be rejoined by the DNA ligase IV/XRCC4 heterodimer (Lees-Miller and Meek, 2003;Spagnolo *et al.*, 2006).

Mutations in any one of these NHEJ components leads to impaired dsb repair and increased sensitivity to ionizing radiation (Zhu *et al.*, 1996;Gu *et al.*, 1997;Gao *et al.*, 1998). DNA-$PK_{cs}$, like Atm and Atr, is a phosphoinositol-3 kinase (PI3K)-related protein kinase (PIKK). DNA-PK can phosphorylate p53

*in vitro* (Lees-Miller *et al.*, 1992), but the role of p53 and apoptosis in the radiosensitive phenotype conferred by DNA-PK mutation is unclear. The finding of a synthetic lethal interaction between DNA-$PK_{cs}$ and Atm provided a clue to the mechanism by which DNA-PK impinges on cell death pathways. *Scid/scid* mice, which lack DNA-PK activity due to mutation in DNA-$PK_{cs}$ (Blunt et al., 1996), and *Atm* null mice are born at normal frequencies, while s*cid/scid Atm-/-* compound mutant embryos die very early in development (Gurley and Kemp, 2001). This shows that Atm function is required for survival of cells with loss of NHEJ capacity. Since p53 is a direct target of Atm signaling, we asked whether simultaneous loss DNA-$PK_{cs}$ and p53 synergized to affect cell death pathways. *Scid/scid p53-/-* mice are viable (Gurley et al., 1998). However, here we report that while cells from *p53* null mice are resistant to ionizing radiation (IR) induced apoptosis, s*cid/scid p53-/-* compound mutant cells are highly sensitized to apoptosis. This identifies a role for DNA-PK in suppression of p53 independent apoptosis and identifies a cellular mechanism by which loss of DNA-PK sensitizes cells to the lethal effects of radiation.

**RESULTS**

As DNA-PK can phosphorylate p53 *in vitro*, we first asked if p53 induction or apoptosis were impaired in the absence of DNA-PK activity. Epithelial cells within the crypts of the small intestine from *SCID, DNA-$PK_{cs}$-/-, Ku70-/-,* and *Ku80-/-* null mice showed no apparent defect in IR-induced p53 expression or apoptosis. 4 Gy whole body radiation induced apoptosis to a similar extent in both wild type and all four DNA-PK deficient strains (Fig 1A,D), and in some cases, the apoptotic response in the absence of DNA-PK was enhanced over the wild type response. *p53-/-* mice showed little or no increase in this early wave of apoptosis, as previously reported (Merritt *et al.*, 1994). The number, staining intensity, and localization of cells that stained for nuclear p53 following radiation were also equivalent between wild type and DNA-PK mutant mice (Fig 1A). Colonic crypt epithelial cells and

hair follicle epithelial cells from dorsal skin of *DNA-PK$_{cs}$* null mice were also sensitive to IR-induced apoptosis, while these same cell types from *p53* null mice were resistant (data not shown). To determine if DNA-PK was required for apoptosis in tumor cells, we crossed SCID mice to *Apc$^{Min}$* mutant mice, which spontaneously develop intestinal adenomas (Su et al., 1992). Intestinal adenomas that developed in *scid/scid Apc$^{Min}$* mice also showed a rapid apoptotic response to IR, equivalent to that seen in tumors from *Apc$^{Min}$* mice with intact DNA-PK (Supplemental Fig 1). T cell lymphomas that developed in SCID mice also showed a robust apoptotic response to DNA damage (Gurley et al., 1998). These *in vivo* results, obtained from multiple normal and neoplastic cell types, using two mutant alleles of *DNA-PK$_{cs}$*, as well as all three components of the DNA-PK holoenzyme, demonstrates that DNA damage-induced p53 activation and apoptosis does not require DNA-PK activity.

DNA-PK deficient cells are highly sensitive to radiation, and apoptosis is one factor that can contribute to radiosensitivity. In contrast, p53 deficient cells are strongly resistant to apoptosis and somewhat radioresistant (Gudkov and Komarova, 2003). To determine if DNA-PK and p53 interact genetically in the radiation response, we examined apoptotic sensitivity in DNA-PK p53 compound mutant mice. Strikingly, the apoptotic response in these double mutants was quantitatively similar to wild type, indicating that the apoptotic defect of p53 null cells was nearly completely rescued by concomitant mutation of DNA-PK. At 4 hours post 4 Gy, the number of apoptotic cells per crypt in *scid/scid p53-/-* mice was similar to *scid/scid* and substantially greater than *p53-/-* mice or unirradiated wildtype mice (Fig 1 and Supplementary Table I). On a per cell basis, mutation in DNA-PK increased the apoptotic sensitivity of p53 null cells ~40 fold. Apoptotic cells were identified by the morphological criteria of cytoplasmic shrinkage, chromatin condensation, and nuclear fragmentation, and confirmed by TUNEL (Fig 1A) and active caspase 3 staining (Fig 2). *p53* null mice remained resistant to rapid onset apoptosis at doses as high as 20 Gy (not shown). Time course analysis showed that apoptosis was

slightly delayed in *scid/scid p53-/-* mice (compare the 2 h time point in Fig 1C) compared to wild type mice and that p53 null mice showed a slight increase in apoptosis at later time points of 24 and 72 h.

To exclude the possibility that the mutant DNA-$PK_{cs}$ protein in SCID mice acts in a gain of function manner to activate apoptosis, we next examined *DNA-$PK_{cs}$* knockout mice. *DNA-$PK_{cs}$-/- p53-/-* compound null mice were also highly sensitized to apoptosis (Fig 1). Rescue of the apoptotic defect of *p53* null cells by mutation of *DNA-$PK_{cs}$* was seen on C57BL/6, 129 x C57BL/6, and C3H x C57BL/6 genetic backgrounds (data not shown) demonstrating this phenotype is independent of strain background. We also observed a *DNA-$PK_{cs}$* gene dosage effect on p53 independent apoptosis, as the number of apoptotic cells per crypt in irradiated *scid/+ p53-/-* mice (0.65 ± 0.3) was six fold greater than in littermate control *p53-/-* mice (0.1 ± 0.01) (Fig 1D).

In wild type mice, IR-induced apoptosis is not uniformly distributed through the small intestinal crypt, but is localized between cell positions four to ten, which overlaps with the stem cell and proliferative compartment of this tissue (Barker *et al.*, 2008). It was previously shown that the distribution of p53 positive cells precisely overlapped with the distribution of apoptotic cells (Merritt et al., 1994) and Fig 1. This plus the absence of apoptosis in p53 null mice implied that the apoptotic sensitivity of these cells is due to p53 and its regulation. However, analysis of the spatial distribution of apoptotic cells in *DNA-PK-/- p53-/-* mice showed a nearly identical profile as in wild type mice (Fig 1B) indicating the apoptotic sensitivity of these cells in not solely due to p53.

We next used a genetic analysis to address the mechanism of this p53 independent apoptotic response. To determine if this was unique to the loss of DNA-$PK_{cs}$, or if it could be triggered by deficiency in any component of the NHEJ pathway, we used a series of mouse mutants, all of which result in defective DNA dsb repair and radiosensitivity. Both *Ku70-/- p53-/-* and *Ku80-/- p53-/-* mice showed an apoptotic response virtually identical to wild type mice, and again much greater than their *p53-/-* littermates (Fig 1A,D). Therefore, disabling any component of the DNA-PK holoenzyme, Ku70,

Ku80, or DNA-PK$_{cs}$, equally and efficiently sensitizes cells to p53 independent apoptosis. Next, we tested the final component of the NHEJ pathway, Ligase IV. Like DNA-PK, Ligase IV deficiency results in inefficient repair of dsbs, confirmed here by increased p-H2AX staining in irradiated crypt cells (Fig 2A). However, in marked contrast to DNA-PK/p53 deficient mice, intestinal crypt cells from *Lig4-/- p53-/-* mice were resistant to IR-induced apoptosis (Figs 1 and 2). This shows that p53 independent apoptosis is not triggered by deficiencies in all NHEJ components, but is specific to DNA-PK.

Deficiency in the PIKK Atm also confers a radiosensitive phenotype. To determine if loss of Atm engaged p53 independent apoptosis, we compared the apoptotic response of *Atm-/-* and *Atm-/-p53-/-* mice. Intestinal crypt cells from irradiated *Atm-/-* mice showed robust nearly wild type levels of apoptosis, as previously reported (Gurley and Kemp, 2007), while those from *Atm-/- p53-/-* mice were as resistant as *p53-/-* mice (Figs 1D and 2). Therefore, the loss Ku70, Ku80, and DNA-PK$_{cs}$, but not Atm or Ligase IV sensitizes *p53* null cells, indicating p53 independent apoptosis is specifically triggered by the loss of DNA-PK.

We next examined possible mediators of this apoptotic response. The serine/threonine kinase, Chk2 is a central effector in the cellular response to DNA damage (Bartek and Lukas, 2003). Chk2 is phosphorylated at threonine 68 residue by Atm at the sites of dsbs (Shiloh, 2003) and can in turn phosphorylate p53, among other substrates. Chk2 has been shown to modulate apoptosis in p53 deficient cancer cell lines suggesting p53 independent functions (Yang *et al.*, 2002;Stevens *et al.*, 2003;Urist *et al.*, 2004). Radiation induced a rapid increase in phospho-Chk2 (Thr68) in crypt cells that peaked at 2 hours and was already reduced by 4 hours (Fig 2C). Irradiated Chk-2 null tissue showed no staining confirming antibody specificity (Supplemental Fig 2). Irradiated *Atm* null crypts showed reduced levels of pChk2, indicating that Atm regulates Chk2 in this setting (Fig 2). In contrast, high levels of pChk2 staining persisted in *DNA-PK$_{cs}$ -/- p53-/-*, *Ku70-/- p53-/-*, and *Ku80-/-*

*p53-/-* mice through 4 hours and was still detected at 24 hours after irradiation. Prominent phospho-Chk2 staining was not seen in *Lig4-/- p53-/- , Atm-/- p53-/-,* or *p53-/-* mice, genotypes which are resistant to apoptosis. E2F1, PML, and p73 have been proposed as mediators of Chk2 regulated apoptosis. However expression patterns of these proteins in irradiated crypts did not correlate with genotype or apoptosis (data not shown).

We next addressed if p53 independent apoptosis is mediated through the intrinsic apoptotic pathway. This pathway is instigated by activation of BH3 only proteins, leading to Bax and Bak mediated release of cytochrome c from the mitochondria (Danial and Korsmeyer, 2004). Cytosolic cytochrome c induces oligomerization of Apaf1 to form the apoptosome which activates and cleaves pro-caspase 9, the initiator caspase in the intrinsic apoptosis pathway (Riedl and Salvesen, 2007). Active caspase 9 in turn triggers cleavage and activation of the executioner caspase 3, which is common to both intrinsic and extrinsic apoptotic pathways. Staining for cleaved caspases 9 and 3 was clearly detected in crypt cells undergoing apoptosis from both wild type and DNA-PK p53 deficient mice, at levels quantitatively similar to apoptosis, but was not detected in the apoptotic resistant genotypes (Fig 2 and Supplementary Table I). This shows that p53 independent apoptosis, engaged by the loss of DNA-PK is executed through the intrinsic mitochondrial pathway.

The chemotherapy agents etoposide and 5-fluorouracil (5-FU) also induce p53-dependent apoptosis, so it was of clinical interest to determine if the absence of DNA-PK sensitized *p53* null cells to these agents. Treatment of mice with the type II topisomerase inhibitor etoposide induced crypt cell apoptosis at 4 h in wild type, *scid/scid,* and *scid/scid p53-/-* mice but not *p53-/-* mice (Fig 3). In contrast, the antimetabolite 5-FU induced apoptosis (peaking at 24 h) in wild type and *scid/scid* mutant but not from *p53-/-* or *scid/scid p53-/-* mice. Radiation and etoposide are thought to induce apoptosis through the creation of dsbs while 5-FU induces apoptosis through alterations in DNA replication or RNA metabolism (Pritchard *et al.*, 1997). Consistent with this, p-H2AX was prominent in crypt cells

from radiation and etoposide, but not 5-FU treated mice (Fig 3). This shows that loss of DNA-PK does not rescue all modes of p53 dependent apoptosis, but is specific to DNA damage initiated apoptosis.

## DISCUSSION

DNA-PK is not required for the canonical p53 mediated apoptotic response to DNA damage. In contrast, DNA-PK plays an anti-apoptotic role, suppressing a robust apoptotic response that is entirely p53 independent. Using a combination of genetic, chemical, and immunohistochemical analyses we characterized this DNA-PK regulated apoptotic response. p53 induction and apoptosis coincided precisely both spatially and temporally within the intestinal crypt implying that the apoptotic sensitivity of these cells is attributed solely to p53 or its regulation. Apoptosis in *DNA-PK/p53* null mice occurred in the very same cell positions and, excepting a slight delay, with similar kinetics. This, instead, indicates that these cells are intrinsically sensitive, and apoptosis can be triggered by either p53 dependent or p53 independent pathways. This region contains the transit amplifying compartment, responsible for generating many of the differentiated cell types of the small intestine. The existence of redundant pathways to regulate apoptosis in these cells may serve as a failsafe mechanism to delete damaged cells and prevent neoplastic transformation.

Apoptosis in DNA-PK/p53 deficient mice was induced by ionizing radiation and etoposide but not 5-FU, implicating DNA damage as the initial apoptotic trigger. However several lines of evidence indicate that DNA damage *per se* is insufficient to trigger rapid onset p53 independent apoptosis. First, radiation doses as high as 20 Gy failed to induce rapid apoptosis in *p53* null intestinal crypts. Second, the IR induced apoptosis that is seen in *p53* null mice is very modest, markedly delayed (>24 hours after IR), and coincides with resumption of abundant mitotic activity. The apoptotic bodies at these late time points were larger and multinucleate, suggesting they are undergoing G2/M cell death

as an indirect consequence of the radiation damage (Merritt et al., 1997). In contrast, the rapid apoptosis (4 hours after IR) seen in *DNA-PK-/- p53-/-* mice is likely a direct response to IR, arguing that the apoptotic responses in *DNA-PK-/- p53-/-* and *p53-/-* mice are fundamentally different. Third, although Ligase IV and Atm deficiency leads to defective DNA repair and radiosensitivity, crypt cells from *Ligase IV/p53* and *Atm/p53* double null mice remained resistant to radiation induced apoptosis. Finally, the fact that deletion of Ku70, Ku80, or DNA-$PK_{cs}$ all showed a quantitatively equivalent and nearly complete rescue of apoptosis in a *p53* null background implicate a direct role of the DNA-PK holoenzyme in the suppression of DNA damage initiated p53 independent apoptosis. A common phenotype associated with loss of Ku70, Ku80, and DNA-$PK_{cs}$ is the inability to activate the holoenzyme DNA-PK and to process DNA double strand breaks. Ligase IV deficiency also results in unrepaired breaks but with one important distinction: DNA-PK activity would not be impaired and therefore the breaks would be synapsed and processed. Unlike any of the DNA-PK subunits, Ligase IV deficiency leads to embryonic lethality, attributed to massive p53 dependent apoptosis (Frank et al., 2000). This lethality is rescued by p53 deletion as *Ligase IV-/- p53-/-* mice are viable. This critical phenotypic distinction among components of the NHEJ pathway is extended by our studies to differential regulation of p53 independent apoptosis.

p53 independent apoptosis was tightly associated with increased phospho-Chk2 and caspase 9 activation, suggesting that the absence of DNA-PK, either directly through loss of kinase activity, or indirectly through the presence of unprocessed dsbs, leads to hyperactivation of Atm signaling through Chk2 and/or other downstream targets leading to engagement of the intrinsic apoptotic pathway.

**SPECULATION**

DNA double strand breaks activate two mutually exclusive responses, DNA repair and apoptosis. The regulation of the choice between these responses is poorly understood. It is now apparent that DNA-PK plays a central role in both, in that it directly mediates dsb repair and suppresses apoptosis. Its role in tumor suppression (Kemp *et al.*, 1999) or its usefulness as a target in cancer therapy (O'Connor *et al.*, 2007) will likely depend on which of these responses predominate. Enhanced apoptosis in the absence of DNA-PK provides an explanation for the radiosensitive phenotype that has long been associated with DNA-PK. Importantly, p53 is not required for this cell death response, pointing to the NHEJ apparatus as a potential target to sensitize cancer cells.

## MATERIALS AND METHODS

**Mice.** DNA-$PK_{cs}$ knock-out mice on a 129 x C57BL/6 background (Gao et al., 1998) or C57BL/6 ($Prdkdc^{scid}$) mice (Jackson Laboratory) were crossed to C57BL/6 p53 knockout mice (from L. Donehower) to generate *DNA-$PK_{cs}$ -/- p53-/-* or *scid/scid p53-/-* mice and control littermates. *Atm-/-* (Barlow et al., 1996), *Ku80-/-* (Zhu et al., 1996), *Ku70-/-* (Gu et al., 1997), and *Ligase4+/-* (Frank et al., 2000) mice were similarly crossed to *p53* null mice to generate *Atm-/-p53-/-*, *Ku80-/-p53-/-*, *Ku70-/-p53-/-*,and *Ligase4-/-p53-/-* mice. Genotyping was done using established PCR protocols and conditions are available upon request. Six to ten week old mice were given 4 Gy of whole body ionizing radiation using a $Co^{60}$ source and sacrificed at given times post-irradiation. Unirradiated age matched littermate controls were also sacrificed for tissues. Tumor-bearing $Apc^{Min}$ mice (Jackson Laboratory) were irradiated and tissues taken 4 h post IR. For chemotherapy studies, mice were injected with etoposide (40 μg/g, i.p) or 5-FU (40 μg/g, i.p.) and tissues examined at 4 h or 24 h respectively.

**Histology.** Tissues were fixed, processed and stained for p53 (Novocastra CM5), cleaved caspase 9 (Asp 353, Abcam ab52298), cleaved caspase 3 (Asp175, Cell Signaling), phospho-Chk2 (T68, Abcam), and phospho-histone H2AX (S139, Cell Signaling) using a three step streptavidin technique as described (Gurley *et al.*, 2007). Numbers of apoptotic figures or positive staining cells were counted in intestinal crypts of ten 40X fields or in ten 100X fields of intestinal adenomas.

**Supplementary information** is available at *EMBO reports* online

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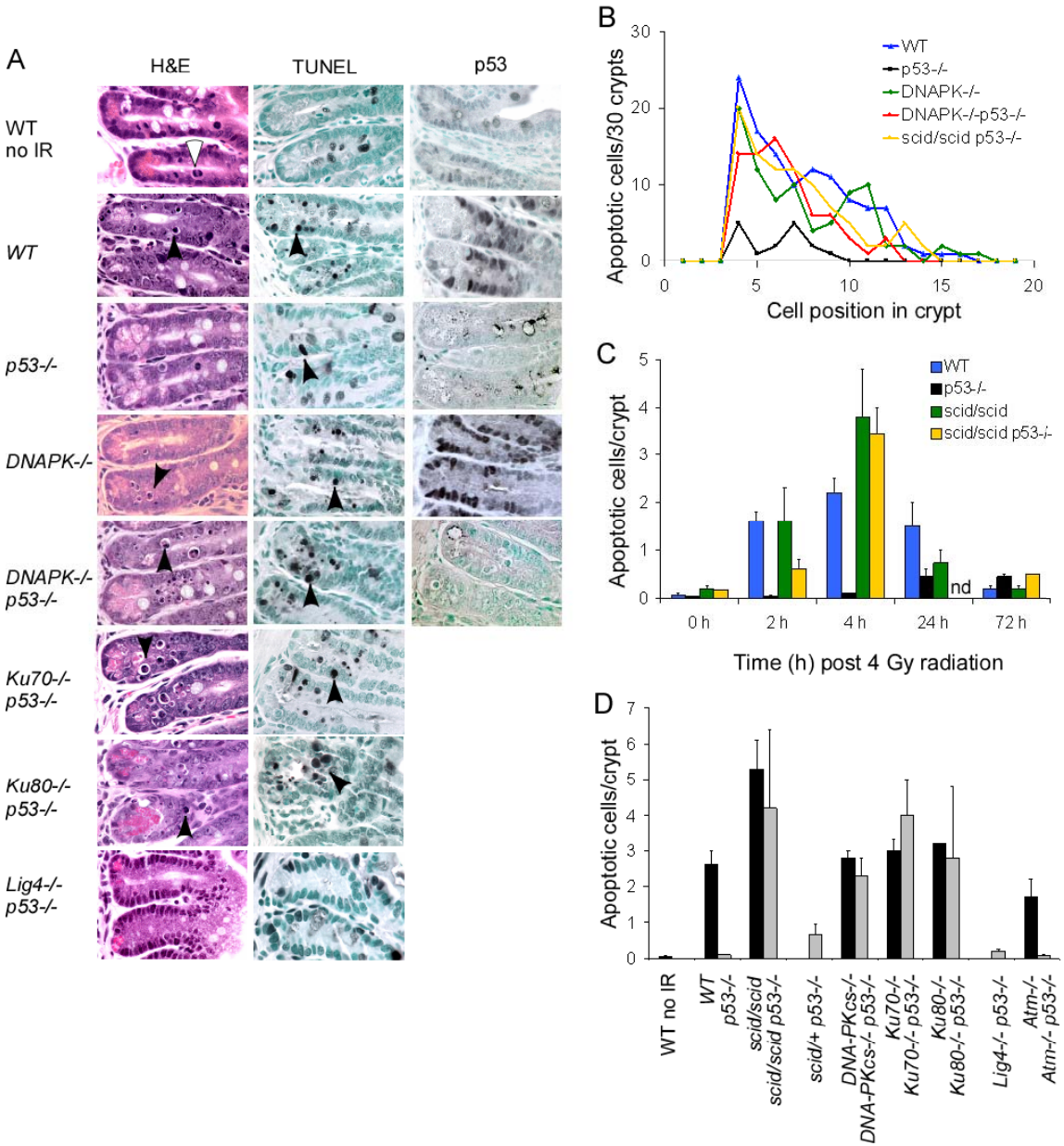
**FIGURE LEGENDS**

**Fig 1 Elimination of DNA-PK rescues the apoptotic defect in p53 null mice.** (A) H&E stained sections of small intestinal crypts from unirradiated (no IR) or irradiated (4 h post 4 Gy) mice. Mitotic cells (white arrowhead) and apoptotic cells (black arrowheads) are shown. Also shown are TUNEL assays and p53 immunostaining. Note spatial coincidence of p53 expressing cells and apoptotic cells. (B) Spatial distribution of apoptotic cells within the crypt. Note the distribution of apoptosis is similar between wild type, *DNA-PK-/-* and *DNA-PK-/-p53-/-* mice, peaking at cell positions 4-7. (C) Time course of small intestinal crypt cell apoptosis after 4 Gy IR in DNA-PK and p53 deficient mice (n.d. = not done). (D) Quantification of apoptotic cells in small intestinal crypts from irradiated mice (4 h post 4 Gy) (ten 40X fields were counted/mouse, ~5 crypts /field and ~50 cells/crypt). Bars are the mean values plus standard deviations from 3-8 mice per genotype except for *Ku80-/-* (n=1).

**Fig 2 Mechanism of apoptosis in DNA-PK and p53 mutant mice**. (A) H&E stained sections and immunostaining for the indicated proteins from intestinal crypts at 4 h post 4 Gy. Note apoptotic cells in WT and *DNA-PK-/- p53-/-* but not *Atm-/- p53-/-, Ligase IV-/- p53-/-* or *p53-/-* mice. Increased p-H2AX is seen in both DNAPK-/- and Lig4-/- crypts but persistent p-Chk2 staining is only seen in *DNA-PK-/- p53-/-* crypts indicating that p53 independent apoptosis correlates with pChk2 activation but not DNA damage per se. Arrowheads point to positive stained cells. (B) Quantification of pChk2 staining in intestinal crypts taken at 4 h post 4 Gy. (C) Time course of pChk2 staining in crypts. Bars are the mean values plus standard deviations from 3-5 mice per genotype except for *Ligase IV-/- p53-/-* (n=2) and *Atm-/- p53-/-* (n=2). Note increased levels and persistent pChk2 staining in scid/scid *p53-/-* mutant mice compared to other genotypes.

**Fig 3 Role of DNA-PK in chemotherapy induced apoptosis**. (A) H&E stained sections and immunostaining for p-H2AX of GI crypts from irradiated (4 h post 4 Gy), etoposide (4 h), or 5-FU (24

h)-treated mice. (B) Quantification of apoptotic cells in GI crypts following treatment with etoposide or 5-FU. Bars are the mean values plus standard deviations from 3-8 mice per genotype.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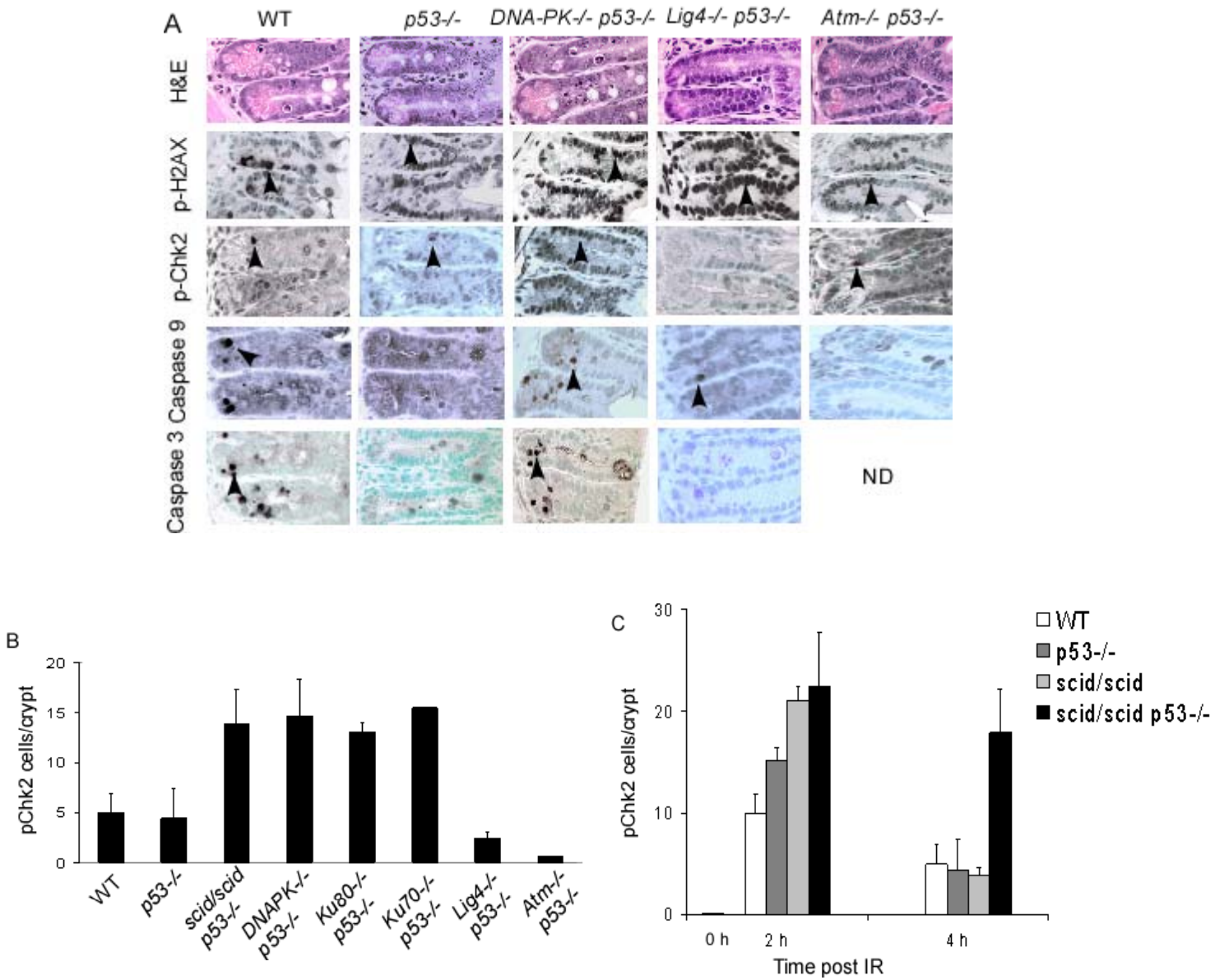
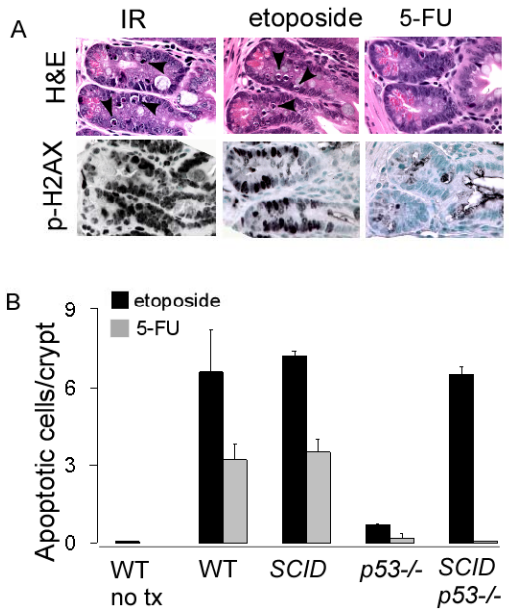
p53-/-
B
Apoptotic cells/30 crypts
Cell position in crypt
WT
p53-/-
DNAPK-/-
DNAPK-/-p53-/-
scid/scid p53-/-
C
Apoptotic cells/crypt
Time (h) post 4 Gy radiation
WT
p53-/-
scid/scid
scid/scid p53-/-
nd
0 h
2 h
4 h
24 h
72 h
D
Apoptotic cells/crypt
WT no IR
WT
p53-/-
scid/scid
scid/scid p53-/-
scid/+ p53-/-
DNA-PKcs-/-
DNA-PKcs-/- p53-/-
Ku70-/-
Ku70-/- p53-/-
Ku80-/-
Ku80-/- p53-/-
Lig4-/- p53-/-
Atm-/-
Atm-/- p53-/-


A
WT
p53-/-
DNA-PK-/- p53-/-
Lig4-/- p53-/-
Atm-/- p53-/-
H&E
p-H2AX
p-Chk2
Caspase 9
Caspase 3
ND
B
pChk2 cells/crypt
20
15
10
5
0
WT
p53-/-
scid/scid p53-/-
DNAPK-/- p53-/-
Ku80-/- p53-/-
Ku70-/- p53-/-
Lig4-/- p53-/-
Atm-/- p53-/-
C
pChk2 cells/crypt
30
20
10
0
0 h
2 h
4 h
Time post IR
WT
p53-/-
scid/scid
scid/scid p53-/-


A
IR
etoposide
5-FU
H&E
p-H2AX
B
etoposide
5-FU
Apoptotic cells/crypt
9
6
3
0
WT
no tx
WT
SCID
p53-/-
SCID
p53-/-