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More efficient repair of DNA double-strand breaks in skeletal muscle stem cells compared to their committed progeny

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Abstract The loss of genome integrity in adult stem cells results in accelerated tissue aging and is possibly cancerogenic. Adult stem cells in different tissues appear to react robustly to DNA damage. We report that adult skeletal stem (satellite) cells do not primarily respond to radiation-induced DNA double-strand breaks (DSBs) *via* differentiation and exhibit less apoptosis compared to other myogenic cells. Satellite cells repair these DNA lesions more efficiently than their committed progeny. Importantly, non-proliferating satellite cells and post-mitotic nuclei in the fiber exhibit dramatically distinct repair efficiencies. Altogether, reduction of the repair capacity appears to be more a function of differentiation than of the proliferation status of the muscle cell. Notably, satellite cells retain a high efficiency of DSB repair also when isolated from the natural niche. Finally, we show that repair of DSB substrates is not only very efficient but, surprisingly, also very accurate in satellite cells and that accurate repair depends on the key non-homologous end-joining factor DNA-PKcs.

Introduction

Stem cells play crucial roles in tissue growth, homeostasis and regeneration. The self-renewal capacity of stem cells and their potential to maintain the tissue depend on their ability to regulate endogenous and exogenous (*e.g.* irradiation) genotoxic stresses. The accumulation of DNA damage and consequent loss of genome integrity due to double strand

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breaks (DSBs) are two of the major causes of apoptosis, senescence and aging, including in stem cells (Lombard et al., 2005; Nijnik et al., 2007; Rossi et al., 2007; Ruzankina et al., 2008). In the small intestine, stem cells at the bottom of the crypt are proliferating and radioresistant, whereas those around the +4 position are quiescent and radiosensitive (Hua et al., 2012; Li and Clevers, 2010; Potten et al., 2009), therefore the response of stem cells to DNA damage can be distinct depending on their origin, cell cycle status, or both. In another report, melanocyte stem cells did not undergo detectable ionizing radiation (IR)-induced apoptosis, but the stem cell niche was depleted due to their differentiation (Inomata et al., 2009).

Inefficient DNA DSB repair can promote genomic rearrangements which can lead to malignant transformations (Reya et al., 2001) thus leading to the notion that stem cells with compromised genome integrity commit altruistic suicide or differentiate, and are more sensitive to DNA damage than other cells. However, hair-follicle-bulge stem cells are resistant to DNA damage-induced apoptosis, largely mediated by higher expression of anti-apoptotic *Bcl-2* (Sotiropoulou et al., 2010). Moreover, highly enriched hematopoietic stem and progenitor cells (HSPCs) express more anti-apoptotic genes (but not *Bcl-2*) and less pro-apoptotic genes (but not *Bim*) than myeloid progenitors (Mohrin et al., 2010). In a context of reduced apoptosis, proficient and accurate DSB repair is necessary to assure that survivor cells do not incur genome instability and deleterious mutations (Chapman et al., 2012). Interestingly, hair-follicle-bulge stem cells display a faster DNA repair than other basal epidermal cells (Sotiropoulou et al., 2010). HSPC cells also display efficient DSB repair but this is frequently associated with genome rearrangements (Mohrin et al., 2010). Adult stem cells and their derived tissues display different sensitivities to radiation-induced DNA damage (Blanpain et al., 2011), suggesting that they might respond differently to genotoxic injury. Unresolved questions include whether other stem cells are simultaneously more apoptosis-resistant and DNA repair prone than differentiated cells, and whether a high occurrence of mutations is the necessary output for efficient DSB repair in stem cells.

Differences in repair efficiency and accuracy could be ascribed to distinct repair mechanisms associated with cell cycle phase. Proliferating cells rely essentially on accurate recombination-dependent repair (HR, homologous recombination), acting mostly during S/G2 (Chapman et al., 2012). In contrast, non-dividing cells rely essentially on non-homologous end-joining (NHEJ), which is active during the entire cell cycle. NHEJ joins the broken ends and displays some inaccuracy depending on the type of DNA ends (Wyman and Kanaar, 2006). In agreement with this notion, quiescent HSPCs express lower levels of HR-associated repair factors and higher levels of NHEJ markers than proliferating HSPCs (Mohrin et al., 2010).

Skeletal muscle growth and regeneration are mediated by satellite (stem) cells that have robust regenerative potential and are quiescent in the adult. After muscle injury, they enter the cell cycle and produce myoblasts that fuse to effect muscle regeneration. Satellite cells subsequently self-renew in their niche, which is located between the myofiber plasmalemma and the basement membrane. Transcription factors including the homeobox/paired domain gene *Pax7*, the myogenic determination genes *Myf5* and *Myod*, and the differentiation gene *Myogenin* play critical roles in satellite cell regulation (Relaix and Zammit, 2012; Tajbakhsh, 2009). The well-defined stages of lineage progression as well as markers and morphological readouts for distinguishing the distinct cell states from stem to differentiated cells provide an ideal system to investigate how stem cells and their progeny in this tissue respond to IR-induced genotoxic stress. Previous studies showed that high-doses of irradiation (18–25 Gy) compromise satellite cell function and muscle regeneration (Boldrin et al., 2012; Gayraud-Morel et al., 2009; Gross and Morgan, 1999; Heslop et al., 2000; Pagel and Partridge, 1999; Wakeford et al., 1991). Muscle regeneration in response to genotoxic stress is affected by a variety of factors, as muscle damage rescues proliferation of some myogenic cells after high doses of limb irradiation (Gross and Morgan, 1999; Heslop et al., 2000). Moreover, preservation of the niche has a key role in muscle regeneration during engraftment (Boldrin et al., 2012), which is also significantly affected by non-myogenic cells like macrophages (Saclier et al., 2013b). Regeneration in normal and irradiated muscle relies on multiple cell types and cell–cell signaling, however the relative contribution of these cells remains unknown. Therefore a systematic analysis of each cell type is critical to understand how regeneration occurs after irradiation. Here, we show that skeletal muscle stem cells exhibit a robust DNA repair machinery, and that they perform IR-induced DSB repair significantly better than their committed progeny, and with a higher accuracy. Further, the proliferation status of cells appears to affect the repair efficiency to a lower extent than does differentiation. Finally, we show that the niche does not significantly affect the repair efficiency of muscle stem cells pointing to a cell autonomous role for DNA repair.

Materials and methods

Ethics statement

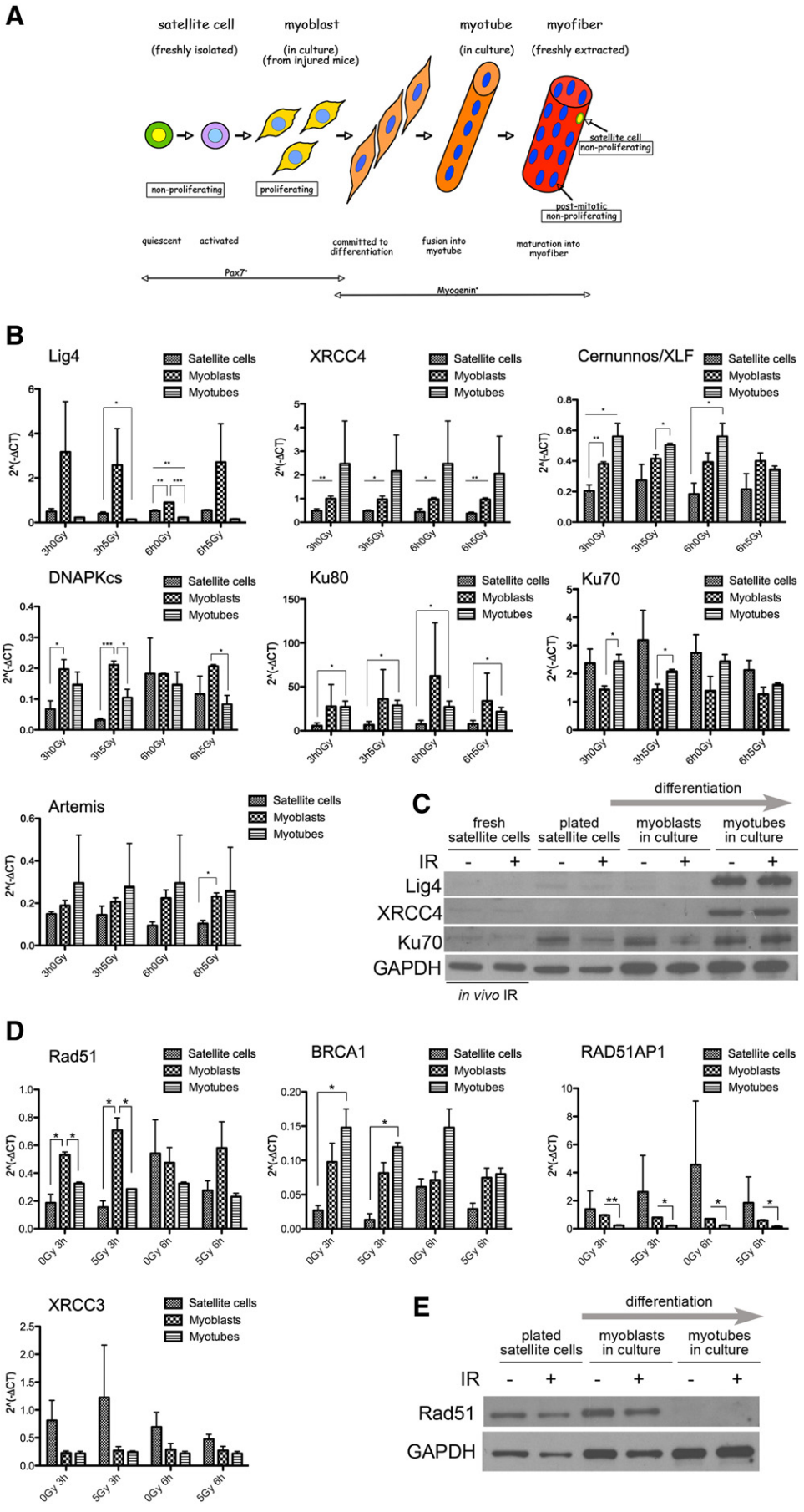
All animal works were performed according to national and European guidelines.

Immunofluorescence and imaging

Cells and myofibers were fixed as described previously (Gayraud-Morel et al., 2007). Cryosections of the TA muscle were obtained and treated as described (Gayraud-Morel et al., 2012). Antibodies and immunocytochemistry reagents are described (Jory et al., 2009). Rabbit polyclonal antibody anti-53BP1 was from Novus Biologicals; anti-phosphohistone H2A.X (Ser139) mouse monoclonal was from Upstate. Images were acquired with a Zeiss Axioplan equipped with an Apotome and an Axiovision software, or a LEICA SPE confocal and LAS software. In isolated cells and fibers, counting of foci was performed on the total cell volume, whereas in muscle cross-sections foci were counted in the plane of section. All images were assembled in Adobe Photoshop or Image J. Some images were assembled as projections of successive confocal acquisitions.

FACS and cell culture

Muscles were dissected and digested in 0.1% Collagenase and 0.25% Trypsin to form a slurry, as described (Gayraud-Morel et al., 2007), that was then sorted by FACS based on GFP


A
satellite cell
(freshly isolated)
myoblast
(in culture)
(from injured mice)
myotube
(in culture)
myofiber
(freshly extracted)
non-proliferating
proliferating
satellite cell
non-proliferating
post-mitotic
non-proliferating
quiescent
activated
committed to differentiation
fusion into myotube
maturation into myofiber
Pax7+
Myogenin+
B
Lig4
XRCC4
Cernunnos/XLF
DNAPKcs
Ku80
Ku70
Artemis
Satellite cells
Myoblasts
Myotubes
2^(-ΔCT)
3h0Gy
3h5Gy
6h0Gy
6h5Gy
C
differentiation
fresh satellite cells
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IR
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differentiation
plated satellite cells
myoblasts in culture
myotubes in culture
IR
Rad51
GAPDH

epifluorescence of *Tg:Pax7-nGFP* mice using FACSAria, BD or MoFlo, and the FACSDiva software. Myoblasts were isolated after injuries performed by intramuscular injections of 10 μl of 10 μg/ml snake venom notexin (Notexin, Lotaxan) on anesthetized mice (0.5% Imalgene/2% Rompun) and TA muscles were collected 5 days post-injury then purified by FACS. For cell culture, cells were plated on Matrigel coated dishes in 1:1 DMEM (Invitrogen) to MCDB201 (Sigma) containing 20% fetal calf serum (FCS) (Invitrogen) and Ultroser (Gayraud-Morel et al., 2007). To allow differentiation, the culture was maintained for 10–11 days. Primary wild type MEFs were cultured in DMEM medium (Dulbecco's modified Eagle's medium, Gibco) supplemented with 10% fetal calf serum (Gibco), 1 mM sodium pyruvate and 50 μg/ml gentamicin at 37 °C with 5% CO_2 and 20% O_2. When indicated, NU7441 (Axon Medchem) dissolved in DMSO was added at a final concentration of 10 μM, and control experiments were performed in the presence of DMSO alone. Cells were pre-treated with NU7441 for 4 h, as described (Smith et al., 2001), and subsequently maintained in a medium containing the drug dissolved in DMSO or an equivalent volume of DMSO for the duration of the experiment.

Single-fiber preparation

Single fibers were prepared as described previously (Gayraud-Morel et al., 2012). The procedure is described in detail in the Supplementary material.

Irradiation

Total body, myofiber, and cell irradiation were performed with a ^{137}Cs Irradiator IBL637 (CIS Biointernational) and occasionally with a Xstrahl RS320 Irradiator. A single irradiation of 5 Gy was delivered, unless otherwise indicated (20 Gy). When indicated, three rounds of 2 Gy each or 5 Gy each were delivered at either day.

RNA extraction, RT and qPCR

Standard RNA extraction and RT-qPCR techniques have been used. They are detailed in the Supplementary material.

Single-cell electrophoresis or comet assay

Pax7-GFP$^+$ satellite cells (untreated muscle) and myoblasts (notexin-injured muscle) from either irradiated (5 Gy) and non-irradiated mice and isolated by FACS 2 h post-IR and immediately used for neutral comet assay (in vivo assay) or, isolated by FACS and then irradiated or not and tested at the indicated times (in vitro assay). For this, cells were embedded in agarose on slides, as previously described (Olive and Banath, 2006). The Olive moment is calculated as Tail DNA (%) × Tail Moment length.

Statistical relevance of observed differences

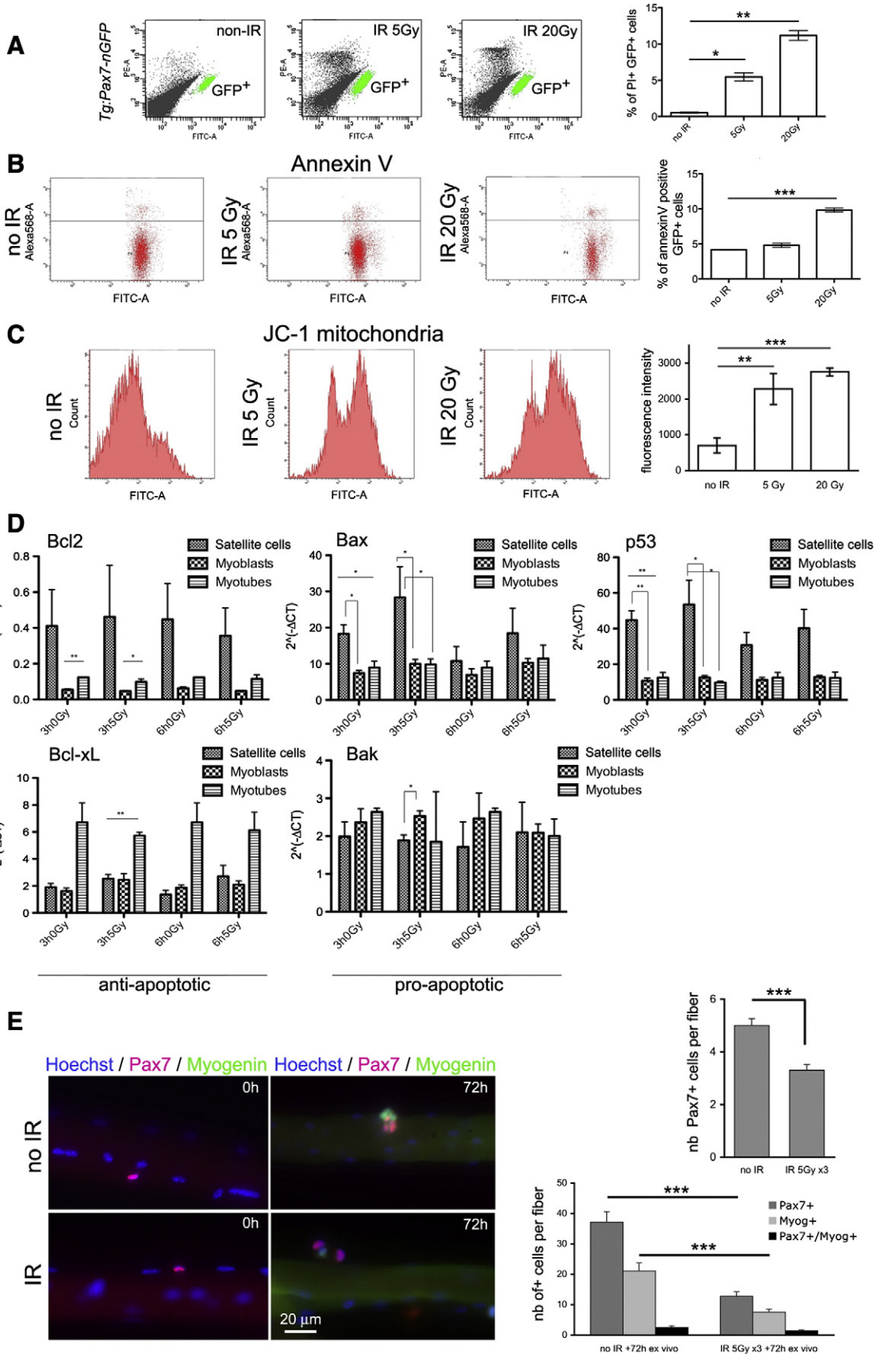
The number of foci in myoblasts, and satellite and differentiated cells on fibers was counted in ≥150 cells in total, originating from 3 to 5 mice. Statistical analysis of two cell populations was performed with the unpaired t test. For qPCR analysis of the expression of a gene in satellites, myoblasts and myotubes, data were first analyzed with the Two-Way ANOVA (with Bonferroni test) using the software Prism; differentiation and irradiation were used as independent variables; and results of this analysis are detailed in the Supplementary material.

Results

NHEJ repair genes are expressed in muscle stem cells and their committed progeny

Given that repair of radiation-induced DSBs in quiescent cells appears to occur primarily by NHEJ (Mohrin et al., 2010; Sotiropoulou et al., 2010), to identify the genes responsible for NHEJ repair in the muscle lineage and whether their expression is activated upon DNA damage, *in vitro* irradiated satellite cells were isolated and compared to myoblasts and differentiated myotubes (Fig. 1A). Transgenic *Tg:Pax7-nGFP* mice permit the isolation of satellite cells by FACS after muscle dissociation (Sambasivan et al., 2009). Key regulators of NHEJ following irradiation include Ku70, Ku80, XRCC4, DNA-PKcs, DNA Ligase4, Cernunnos or XRCC4-like factor (XLF), and Artemis (Wyman and Kanaar, 2006). RT-qPCR of the DNA damage repair (DDR) genes showed that, in spite of their metabolically quiescent state, muscle stem cells transcribe all of the known NHEJ genes (Fig. 1B). Unactivated satellite cells and myofiber nuclei do not divide, and they are therefore expected to rely essentially on NHEJ to repair DSBs. The first division of satellite cells occurs about 20–30 h following injury (Rocheteau et al., 2012), therefore they were not expected to divide under the conditions tested. At 3 h post-IR, NHEJ factors were transcribed at comparable levels as in cultured cells also in

Figure 1 Expression of NHEJ and HR factors in satellite cells and in their progeny. A) Scheme of myogenesis, adapted from (Zammit et al., 2006), indicating key myogenic markers and replicative state of cells. B) RT-qPCR of NHEJ genes. Satellite cells were isolated by FACS from *Tg:Pax7-nGFP* mice and assayed directly (overnight seeding) or cultured to give rise to myoblasts in culture (3 days) and myotubes (10–11 days), n = 3 (myoblasts and myotubes) or n = 5 (satellite cells) mice/condition. Cells were irradiated at 5 Gy (1 Gy ≈ 25–40 DSBs/mammalian cell (Rothkamm and Lobrich, 2003). With the exception of Ligase 4 (Lig4; expressed at higher levels in satellite cells than myotubes), the other NHEJ factors were expressed at higher or at similar levels in both. C) Western blots of key NHEJ factors and reference GAPDH in cells treated as in panel B, and also in satellite cells (first two lanes) freshly isolated from irradiated and non-irradiated mice (3 h post-IR or non-irradiated). D) RT-qPCR of HR genes. E) Western blots of the HR factor Rad51 and reference GAPDH in cells treated as in panel D. See supplementary material for the Two-Way ANOVA analysis. Mean ± SEM; unpaired t tests; *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$.


A
Tg:Pax7-nGFP
non-IR
IR 5Gy
IR 20Gy
GFP+
FITC-A
PE-A
% of PI+ GFP+ cells
no IR
5Gy
20Gy
B
Annexin V
no IR
IR 5 Gy
IR 20 Gy
Alexa568-A
FITC-A
% of annexinV positive GFP+ cells
C
JC-1 mitochondria
Count
fluorescence intensity
D
Bcl2
Bax
p53
Bcl-xL
Bak
Satellite cells
Myoblasts
Myotubes
2^(-ΔCT)
3h0Gy
3h5Gy
6h0Gy
6h5Gy
anti-apoptotic
pro-apoptotic
E
Hoechst / Pax7 / Myogenin
0h
72h
20 µm
nb Pax7+ cells per fiber
IR 5Gy x3
nb of+ cells per fiber
Pax7+
Myog+
Pax7+/Myog+
no IR +72h ex vivo
IR 5Gy x3 +72h ex vivo

satellite cells freshly isolated from irradiated mice (or non-irradiated controls) (Supplementary Fig. S1). Western blot showed lower levels of key NHEJ factors in satellite cells and myoblasts compared to differentiated myotubes (Fig. 1C).

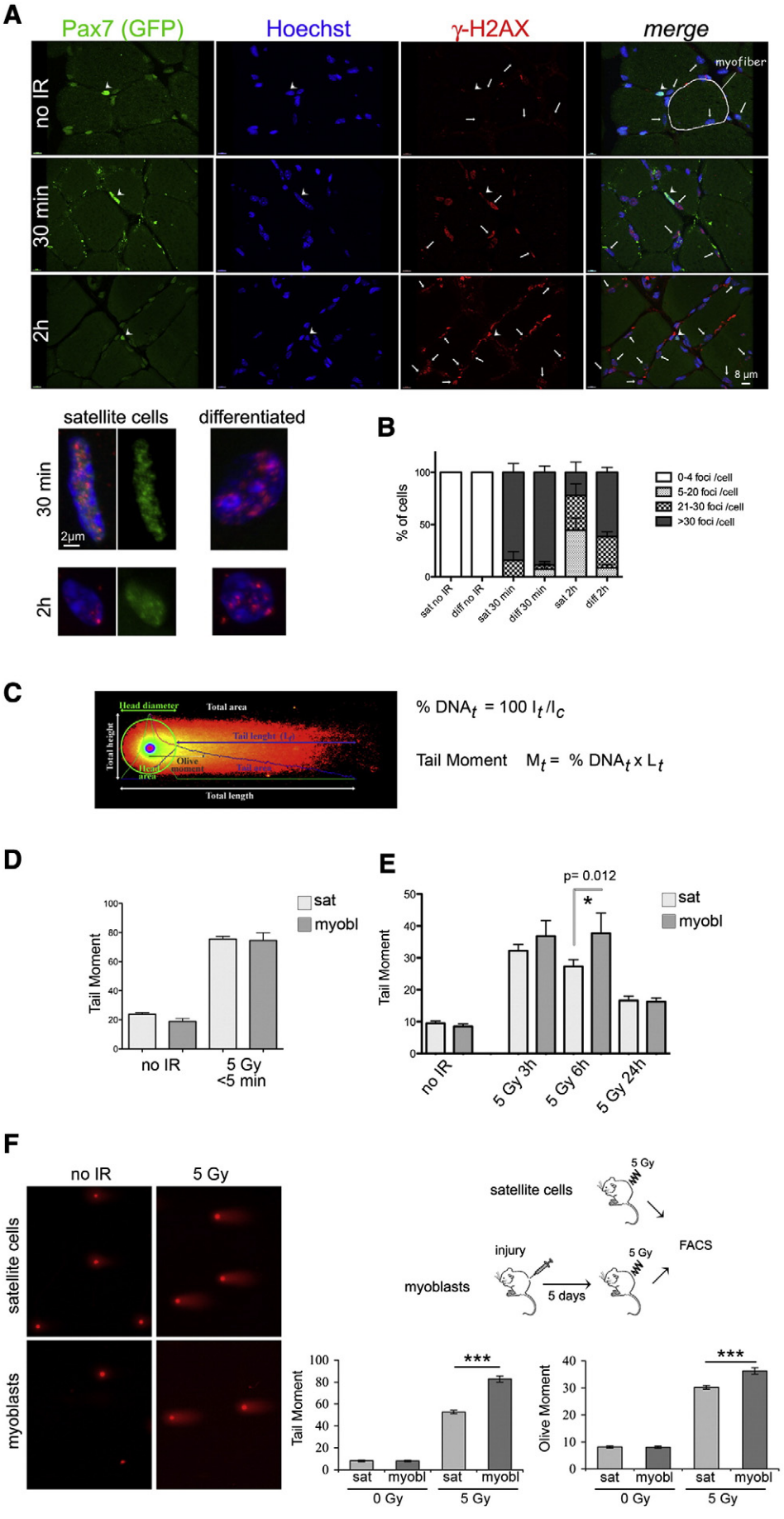
Conversely, proliferating myoblasts, which are expected to rely largely on HR for the repair of DSBs expressed higher levels of the HR factor Rad51 compared to satellite cells and myotubes (Fig. 1D). This result was confirmed by Western blot (Fig. 1E). In addition to HR factors, myoblasts in culture, but not myoblasts isolated from injured mice (either irradiated or not), transcribed key NHEJ factors as non-dividing cells, with protein levels comparable to satellite cells (Fig. 1B and Supplementary Fig. S1). In summary, satellite cells and myoblasts display similarly low levels of NHEJ proteins compared to myotubes, and satellite cells also express HR factors. We note that ionizing radiation *per se* did not promote significant changes in the transcription of the NHEJ genes tested. Therefore, satellite cells and their progeny express NHEJ factors even in the absence of genotoxic stimulus, suggesting that they are proficient in NHEJ.

Muscle stem cells do not undergo significant apoptosis and retain their differentiation potential after IR-induced genotoxic stress

To investigate the effect of IR-induced genotoxic stress on muscle stem cells, we examined several cellular responses including apoptosis, senescence and differentiation. *Tg:Pax7-nGFP* mice were irradiated with either 5 or 20 Gy, and cells isolated by FACS were used to assess the stem cell response to IR. FACS profiles of total muscle cells enriched for the GFP^+ population and stained with propidium iodide (PI) revealed that 60 h post-IR, PI-positive GFP^+ cells accounted for 5.46 ± 0.58% (5 Gy) and 11.17 ± 0.66% (20 Gy) of the GFP^+ population (Fig. 2A), indicating a good survival of satellite cells even at high IR doses. These values were significantly lower than apoptosis detected upon irradiation of myogenic cells in culture (Caiozzo et al., 2010); however in the latter study, the myogenic cells were isolated by selective growth in culture. Subsequent staining of satellite cells with AnnexinV, a marker of apoptosis (Creutz, 1992), revealed low levels of apoptosis in the GFP^+ population of IR-treated mice compared to control non-irradiated mice. Notably, more apoptotic cells were observed in 20 Gy compared with 5 Gy IR and control mice, albeit in all cases over 90% of the cells were AnnexinV-negative (Fig. 2B). Apoptosis was also measured globally on cells extracted from muscle tissue, which includes a small fraction of satellite cells and a large fraction of differentiated and non-myogenic cells. For this, cells extracted from the muscle were stained with the mitochondrial marker JC1, which provides an early indication of the initiation of cellular apoptosis (Salvioli et al., 1997). A significant number of total cells undergo apoptosis post-IR (Fig. 2C). Thus, the relatively high survival rate and low apoptosis of satellite cells following irradiation is surprising given that cells from the total muscle extract displayed a large apoptotic response. Low levels of apoptosis (<2%) were confirmed 24 h post-IR (5 Gy) in satellite cells on cryosections of the *Tibialis anterior* (TA) muscle from irradiated *Tg:Pax7-nGFP* mice (not shown), as well as in satellite cells isolated from irradiated mice (not shown), and immunostained with anti-cleaved Caspase-3.

An analysis of the anti-apoptotic (*Bcl2*, *Bcl-xL*) and proapoptotic (*Bax*, *Bak*) genes (Cory et al., 2003) showed marked differences in their expression among muscle stem cells and their committed progeny (Fig. 2D). Notably, *Bcl2* was expressed the highest in satellite cells, in spite of high levels of its negative regulator *p53* (Mirzayans et al., 2012) and of the p53-target gene and *Bcl2*-antagonist *Bax*. Interestingly, satellite cells also displayed a proapoptotic signature compared to differentiated myotubes, where low levels of expression of *Bcl-xL* and high levels of *Bax* were also observed, which

Figure 2 Evaluation of the cellular response of muscle stem cells to irradiation. A) FACS profiles of control, 5 Gy and 20 Gy IR *Tg:Pax7-nGFP* mice; satellite cells were assayed 60 h after IR and compared with non-IR controls (n = 3 mice/condition). FACS profiles of total myogenic cells enriched for GFP^+ population and percent of PI-positive GFP^+ cells (mean ± SEM). B) GFP^+ cells were assayed for apoptosis (FACS AnnexinV-Alexa568 treated GFP^+ cells, percent Annexin-positive GFP^+; mean ± SD). Red (apoptosis) fluorescence and green (GFP, satellite cells) fluorescence indicated. For AnnexinV control, satellite cells were cultured, treated 4–6 h with H_2O_2 (1 mM) to induce apoptosis, and the gate was adjusted for AnnexinV positivity (control: 12.6%; H_2O_2: 87.4%, not shown). C) Muscle extracts from control, 5 Gy and 20 Gy irradiated mice (harvested 60 h post-IR; see panel A) were treated with JC-1 (Cayman's JC-1 Mitochondrial Membrane Potential Assay Kit) which measures changes in mitochondrial membrane potential, $\Delta\psi m$, a critical parameter of mitochondrial function used as an indicator of cell health (healthy cells, red; apoptotic cells, green). Representative plots of *Tg:Pax7-nGFP* mice shown for control, IR 5 Gy, and IR 20 Gy mice (n = 3 mice/condition). Green fluorescence values shown on right panel; mean ± SD. Mann–Whitney test, *** $p \leq 0.001$. D) RT-qPCR of pro-apoptotic and anti-apoptotic genes involved in cell survival. Freshly isolated satellite cells (overnight seeding) compared to cultured myoblasts (3 days) and myotubes (10–11 days); n = 4 for satellite cells (but *Bax* n = 3) and myotubes; n = 3 for myoblast (but n = 2 for 0 Gy 6 h) condition. E) Wild-type mice were exposed to 3 rounds of 5 Gy IR (individual sublethal dose) at two-day intervals and sacrificed after 22 days. Myofibers were stained with anti-Pax7 and anti-Myogenin antibodies (left panels). Satellite cells were enumerated on freshly isolated EDL myofibers stained for Pax7 (top histogram) and Myogenin (<3% positive cells in both conditions, data not shown). Continuous differentiation and fusion with myofiber might occur after IR, however, centrally located myonuclei (hallmark of newly fused cells) were not noted. Unaffected differentiation could be due to satellite cells remaining in niche with unrepaired DSBs, which would then undergo mitotic checkpoint arrest upon activation and enter apoptosis. To test for this, cell division, differentiation ($Myogenin^+$) and self-renewal ($Pax7^+$) were assessed (Zammit et al., 2004) in niche associated satellite cells from control and irradiated (3 × 5 Gy) mice incubated without attachment for 3 days (right lower panel) and in isolated satellite cells in culture for 4–7 days (Supplementary Fig. S2C; data not shown). IR-treated mice or isolated satellite cells exhibited all of these properties, confirming no change in cell fate upon IR. Unpaired t tests; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.


A
Pax7 (GFP)
Hoechst
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merge
no IR
30 min
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myofiber
8 µm
satellite cells
differentiated
30 min
2h
2µm
B
% of cells
100
50
0
0-4 foci /cell
5-20 foci /cell
21-30 foci /cell
>30 foci /cell
sat no IR
diff no IR
sat 30 min
diff 30 min
sat 2h
diff 2h
C
Head diameter
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Total height
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Head area
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D
Tail Moment
sat
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no IR
5 Gy <5 min
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p= 0.012
*
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5 Gy 3h
5 Gy 6h
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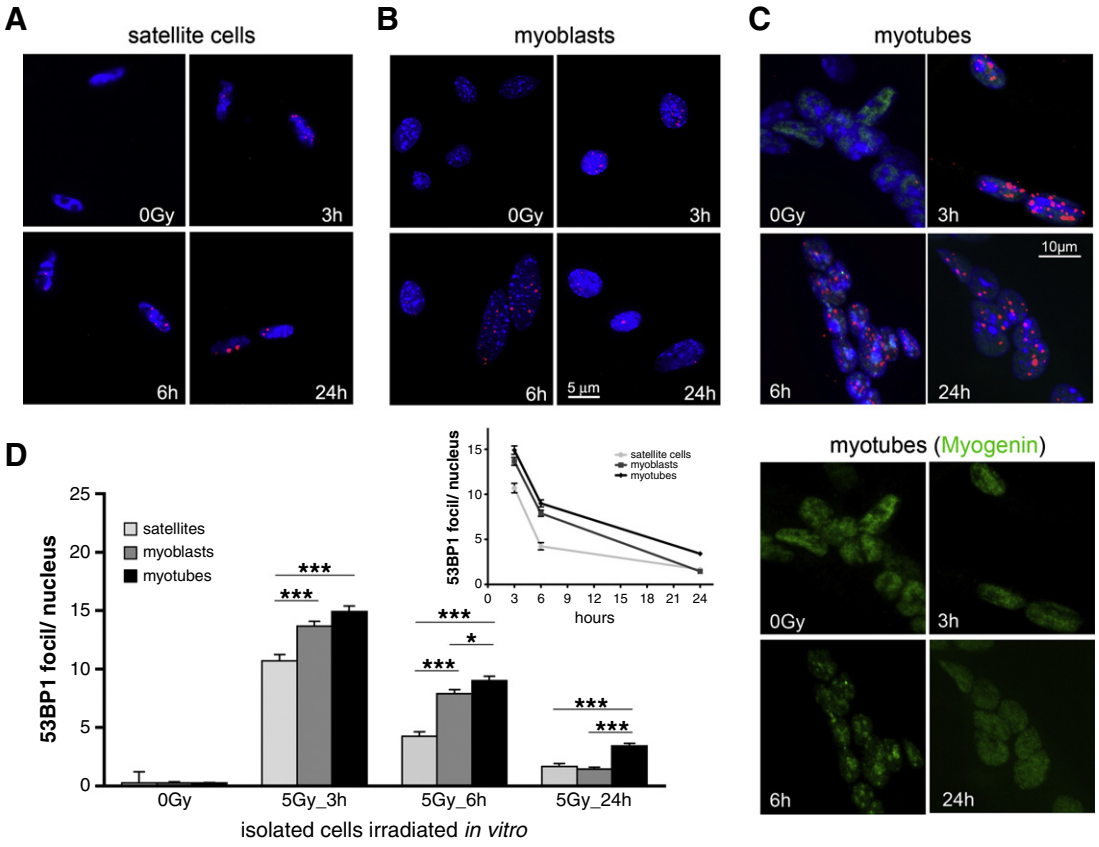
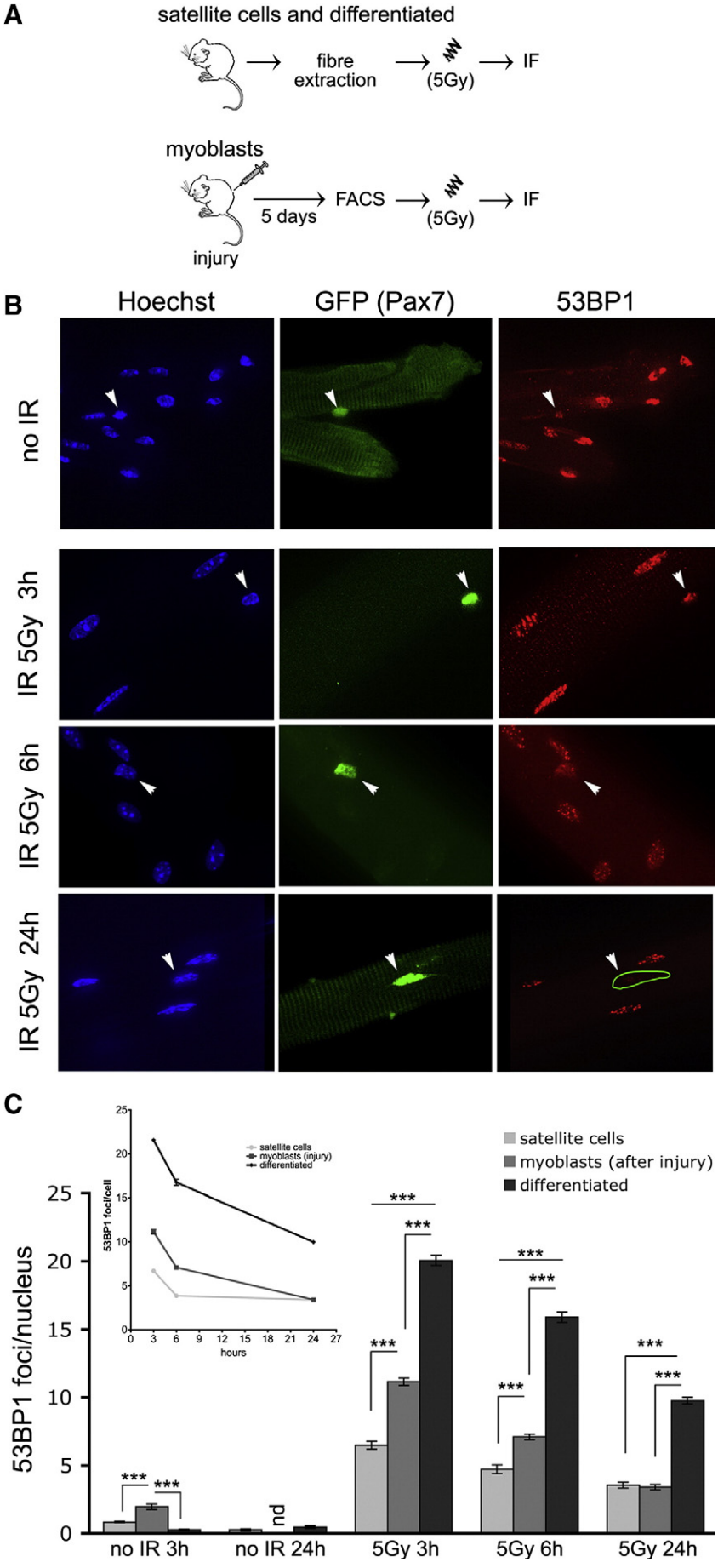


Figure 4 More efficient repair of DSBs in muscle satellite cells than in myoblasts and myotubes in culture. A) Satellite cells were isolated by FACS, plated for 12–14 h, exposed to 5 Gy IR, or cultured to give rise to B) myoblasts in culture (3 days) and C) myotubes (10–11 days) then irradiated, and harvested at indicated times post-IR. n = 3 mice/condition. Sites of DSB repair were revealed by anti-53BP1 (red) and nuclei with Hoechst; panels below in C are immunolabeled with differentiation marker Myogenin (green). D) Enumeration of 53BP1 foci at time intervals after irradiation of satellite cell and committed progeny in culture. Mean ± SEM; unpaired t test (*p ≤ 0.05, ***p ≤ 0.001). Insets, number of foci/nucleus as a function of time post IR. DSB repair kinetics appeared bi-phasic for satellite cells and their progeny, with a more rapid phase before 6 h. For satellite cells and myoblasts compare these kinetics with those of residual DNA damage in Fig. 3E.

promote alternative complexes with cytoplasmic p53 to block or promote, respectively, apoptosis (Chipuk et al., 2005). This pro-apoptotic signature may be linked to the distinct basal expression of p53 in satellite cells (Fig. 2D), confirmed by immunofluorescence (Supplementary Fig. S2A). Indeed p53, a mediator of DNA repair, growth arrest, and apoptosis (Kruse and

Figure 3 Occurrence of DNA damage in satellite cells, myoblasts and differentiated cells upon irradiation. A) Muscle cross-sections from TA muscle extracted from *Tg:Pax7-nGFP* non-irradiated and irradiated (5 Gy) mice 30 min and 2 h post-IR. Upper panels, muscle cross-sections immunostained with anti-GFP (for Pax7-GFP), anti-γH2AX, and Hoechst for nuclei. Anti-GFP and merge panels are intentionally overexposed for the 488-fluorescence to identify the individual myofibers (upper right panel, contour of one myofiber). Arrowhead, satellite cells (Pax7-GFP$^+$); arrow, differentiated nuclei. Lower panels, satellite cells (Pax7-GFP$^+$) and myonuclei (Pax7-GFP$^-$) at higher magnification. Cells from sections at 30 min and 2 h post-IR are shown. B) Percent of satellite (sat) and differentiated (diff) cells with indicated number of γH2AX foci. Irradiated (n = 20 for each time point) and non-irradiated (n = 30) satellite cells, and irradiated (n = 27 for 30 min, n = 100 for t = 2 h) and non-irradiated (n = 35) differentiated nuclei were analyzed from 8 (no IR) and 16 cross-sections (30 min and 2 h), and n = 2 mice. C) Upper panel: schematic of key parameters for analysis of DSBs by neutral comet assay. D) Tail moment of non-irradiated and irradiated satellite cells and myoblasts (from injury) immediately (<5 min) after irradiation (n = 3 mice/condition); Mann–Whitney test. E) Tail moment of satellite cells and myoblasts at different time points following IR *in vitro* (n = 3 mice/condition) Unpaired t test. F) Left panels: Microscopic fluorescent view of freshly isolated satellite cells and myoblasts (from injured mice) irradiated *in vivo* or non-irradiated submitted to an electrophoretic field, 2 h post-IR (5 Gy). The scheme on the right corresponds to the treatment of hindlimbs in each mouse. Irradiated satellite cells and myoblasts exhibit significant migration of damaged DNA compared to non-irradiated cells, resulting in high values of the comet Tail moment and Olive moment compared to cells from non-irradiated mice, as expected. Panels on the lower right show quantification of Tail moment and Olive moment in all conditions tested (n = 300 cells/condition, n = 2 mice). Unpaired t test; ***p < 0.001.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B
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53BP1
no IR
IR 5Gy 3h
IR 5Gy 6h
IR 5Gy 24h
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satellite cells
myoblasts (after injury)
differentiated
53BP1 foci/nucleus
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nd
no IR 3h
no IR 24h
5Gy 3h
5Gy 6h
5Gy 24h

Gu, 2009), is also a key regulator of muscle stem cell number (Schwarzkopf et al., 2006). Interestingly, transient increased levels of p53 upon irradiation was observed by immunofluorescence in satellite cells, indicating that the protein is stabilized in these cells after genotoxic stress, whereas p53 levels remained lower in differentiated myonuclei.

We then determined whether IR affected satellite cell motility and proliferation. Live videomicroscopy showed comparable cell motility and proliferation of irradiated satellite cells compared to control cells (data not shown). Moreover, the number of differentiated cells determined by immunofluorescence with the upstream marker Pax7 and the differentiation marker Myogenin showed rare Myogenin$^+$ cells (<3%) on freshly isolated myofibers from irradiated mice (3× 5 Gy) and non-irradiated controls (Fig. 2E, left panels). These data suggest that differentiation is not a major response to IR for muscle stem cells. In contrast, the number of Pax7$^+$ satellite cells decreased post-IR (Fig. 2E, right upper panel); however, at lower doses (5 Gy; 3× 2 Gy), the number of satellite cells/ fiber was not affected (Supplementary Fig. S2B). Cell division, differentiation (Myogenin$^+$) and self-renewal (Pax7$^+$) were maintained also in isolated myofibers irradiated *in vitro* and incubated in culture (Zammit et al., 2004) (Fig. 2E, right lower panel; data not shown). Accordingly, newly formed myoblasts and differentiated myotubes were observed 3-7 days after plating of muscle stem cell isolated from irradiated mice (Supplementary Fig. S2C; data not shown). Once normalized to the original number of cells/fiber, after 72 h *ex vivo* Pax7$^+$ cells increased in number by 7.4-fold when originating from non-irradiated mice and 3.8-fold from irradiated mice, suggesting a long-term decrease in satellite cell renewal potential after irradiation *in vivo*. Further, twice as many Myogenin$^+$ differentiated cells were obtained from non-irradiated mice compared to those obtained from irradiated mice. Thus, at certain doses, irradiation affects satellite cell number and differentiation potential post-IR.

Occurrence of DNA damage and its repair in myogenic cells shortly after irradiation

The presence of DNA damage in the muscle was assessed on cryosections of the TA muscle from irradiated *Tg:Pax7-nGFP* mice, and immunostained with anti-γH2AX (Fig. 3A, upper panel). Histone H2AX is phosphorylated at S139 (γH2AX) in the presence of DSBs, and is used as a general marker of DSBs (Rogakou et al., 1998). Cells were divided into subpopulations according to the number of foci/cell (Fig. 3B). Fig. 3B shows that 30 min post-IR the large majority of satellite cells and differentiated myonuclei contained >30 foci indicating extensive and comparable levels of DNA damage in both cell states. However, 2 h post-IR satellite cells had less foci/cell compared to differentiated myonuclei, suggesting that the repair of DSBs was faster in the former. Moreover, using single-cell electrophoresis (Fig. 3C) in neutral conditions, we assessed the occurrence of DSBs. Freshly isolated satellite cells and myoblasts (from injured mice) showed similarly high levels of DNA damage immediately after IR, as measured by comet Tail moment (Fig. 3D). Moreover, following overnight plating, satellite cells displayed lower levels of DNA damage than myoblasts 6 h post-IR, indicating faster repair during this period (Fig. 3E). By 24 h, however, myoblasts had also repaired DNA damage as much as satellite cells. Further, in cells isolated by FACS after irradiation *in vivo*, 2 h after irradiation the extent of DNA damage measured by comet Tail moment and Olive moment was lower in satellite cells than in myoblasts (Fig. 3F). Therefore, both electrophoresis of single cells and assessment of DSBs in muscle sections *in vivo* indicate similar levels of DNA damage upon irradiation in satellite *vs.* myoblasts or differentiated cells, and suggest a more rapid repair of DSBs in satellite cells than in myoblasts or differentiated myonuclei.

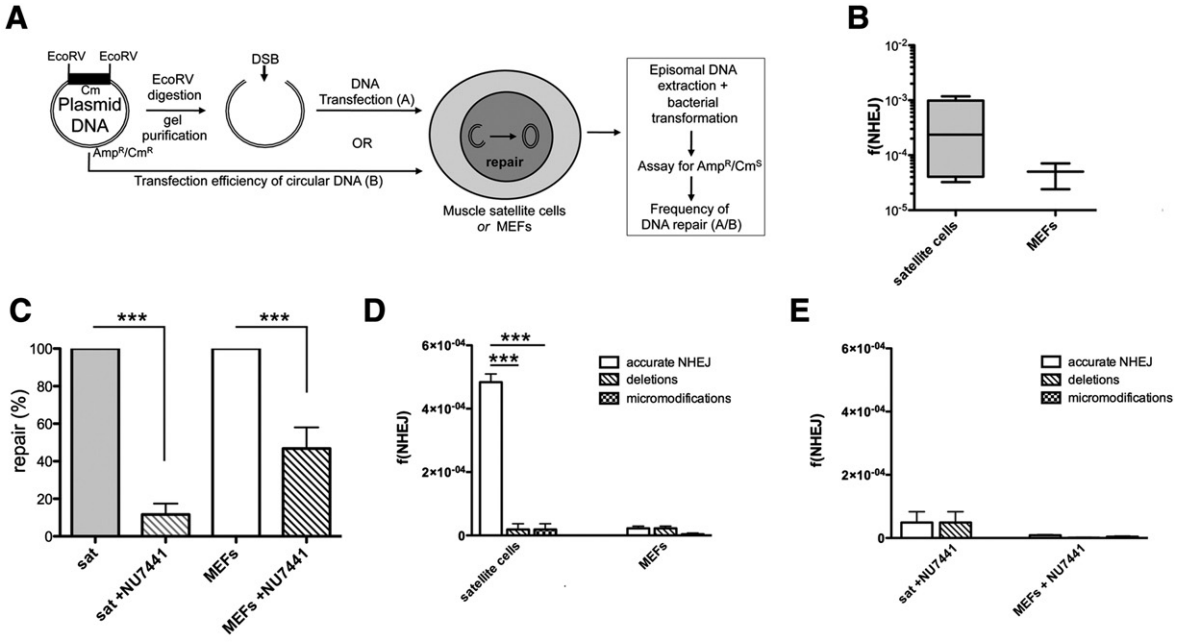
By immunofluorescence of pATM (phosphoS1981), we also observed activated DNA damage response in satellite cells and differentiated myonyuclei within the myofiber upon irradiation (Supplementary Fig. S3B). We then assessed the efficiency and kinetics of DSB repair in satellite cells, using high-resolution imaging and statistical analysis of DSB nuclear markers in single cells (Chayot et al., 2010a). Enumeration of γH2AX and 53BP1 (a DDR factor that is retained at DSBs) foci loss by immunofluorescence has been successfully employed to measure DSB repair upon genotoxic stress (Noon et al., 2010). We found that at short time post-IR satellite cells displayed robust DSB repair either as isolated cells or within the myofiber (Supplementary Figs. S3A, D). As γH2AX is not fully specific for DSB (Marti et al., 2006) and is also sensitive to chromosomal environment (Seo et al., 2012) for the next experiments we used 53BP1 as a DSB immunomarker.

Isolated muscle stem cells repair IR-induced DSB more efficiently than their differentiated progeny

We tested DSB repair at longer times post-IR, under conditions compatible with all experimental systems used in this study

Figure 5 Efficient repair response to IR-induced DSBs in muscle stem cells in the myofiber niche *in vitro*. A) Scheme of *in vitro* experiments. Immunoflourescence (IF). B) Freshly isolated EDL myofibers from *Tg:Pax7-nGFP* mice exposed to 5 Gy IR; DSB foci were enumerated (n = 5 mice) using anti-53BP1 staining. White arrowheads, satellite cells (anti-GFP antibody). C) Histogram of number of 53BP1 foci after IR. *Per* condition, 150–200 satellite cells and differentiated myonuclei were counted, from 75 myofibers (n = 4 mice). Myoblasts from *Tg:Pax7-nGFP* injured mice were isolated by FACS, irradiated or non-irradiated and fixed at indicated time points (300 cells analyzed/condition; n = 3 mice). Average number of 53BP1 foci in differentiated myonuclei was significantly higher (3-fold at 3 h post-isolation) than in satellite cells. From 3 to 24 h post-IR, satellite cells had an average of 4–7 foci/nucleus; differentiated nuclei had 10–20 foci/nucleus (n = 5 mice). Myoblasts had 72% and 50% more foci than satellite cells at 3 h and 6 h, respectively. Residual DNA damage was noted at 24 h, with satellite cells and FACS-isolated myoblasts from injured mice displaying an average of ≤4 foci/nucleus, whereas differentiated cells in fibers retained more than 10 foci. In the absence of irradiation, myoblasts displayed a higher background level of 53BP1 foci than the other nuclei, probably due to DNA damage produced during DNA replication (Branzei and Foiani, 2010; Lukas et al., 2011). Myoblast immunolabeling not shown in panel A. Mean ± SEM. Unpaired t test, ***p < 0.001 Nd, not done. Insets, number of foci/nucleus as function of time post IR.

(see next sections), and where differences in repair kinetics were assessed by enumeration using DSB markers. In the first of three experimental paradigms, the kinetics of DSB repair was determined in freshly isolated satellite cells, myoblasts (cultured for 3 days), and myotubes (cultured for 10–11 days). Although non-irradiated control cells showed little to no 53BP1 foci (~1–3/nucleus), all cell states examined exhibited between 10 and 15 nuclear foci 3 h after 5 Gy IR (Fig. 4). Interestingly, at 3 h and 6 h after IR, satellite cells had significantly fewer 53BP1 foci compared to myoblasts and myotubes (n = 3 animals). In addition, the decline in number of foci was greater in satellite cells than in myoblasts and in myotubes. By 24 h all cells retained some unrepaired DNA damage, indicating persistence of DNA damage, however in



F

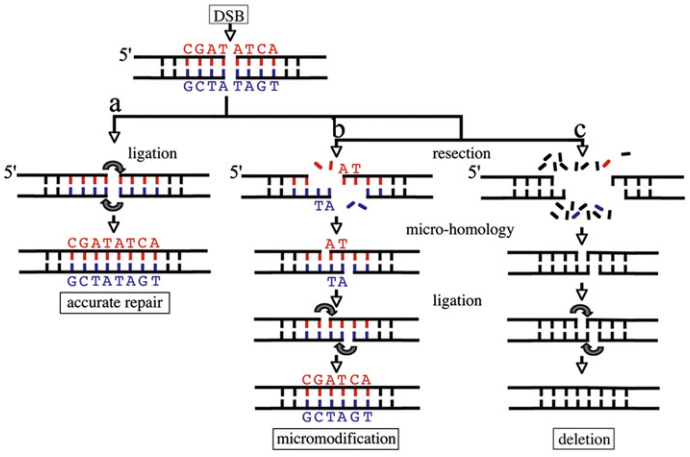
EcoRV

```
5'  TCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAC  3'
3'  AGGGGGCCCGACGTCCTTAAGCTATAGTTCGAATAGCTATGGCAGCTG  5'
```

Clone	MH	5'Δ				3'Δ
Satellite cells						
S66	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
S64	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
S165	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
S31	0	0	TCCCCCGGGCTGCAGGAATTCGAT		TGGGCC	-771
S150	6	-136	AACAATT		CACTGG	-77
S172	nd	nd			CCGCTA	-477
MEFs						
M3	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
M122	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
M123	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
M29	2	-45	GGTGGC		GGCCCG	-34
M30	4	-49	CCGCGG		TGGCGT	-117
M20	1	-166	AGCTCC		TTGCGC	-211
M12	1	-268	ATTTTG		GACACG	-87
M4	nd	-571	TCCACC		nd	nd

Clone	MH	5'Δ	+ NU7441			3'Δ
Satellite cells						
SN6		-687	GGCGGC		AATTTA	-42
MEFs						
MN4	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
MN12	2	0	TCCCCCGGGCTGCAGGAATTCGAT	CAAGCTTATCGATACCGTCGAC		-2
MN7	1	0	TCCCCCGGGCTGCAGGAATTCGAT	ACCGTCGAC		-15

G



satellite cells and myoblasts this number was slightly higher than background levels, whereas 3.4 ± 0.2 foci persisted in myotubes.

These observations indicate that muscle stem cells repair radiation-induced DSBs more efficiently than their committed progeny in culture. They also show that proliferating cells repair DSBs less rapidly than quiescent cells. Moreover, the absence of proliferation is associated with either high or low repair efficiency (satellite cells and myotubes, respectively), suggesting that the proliferation state might not be the major determinant in the efficiency of DSB repair.

Muscle stem cells repair IR-induced DSB more efficiently than their differentiated progeny in myofibers *in vitro* and *in vivo*

Next we investigated DSB repair on isolated myofibers where muscle stem cells remain associated with their niche and where the quiescent and differentiated states reflect cell cycle exit at the time of isolation. We showed that at short times post-IR γH2AX signal decreases more rapidly in satellite cells than in differentiated cells (see Supplementary Figs. S3C, D). Fig. 5 shows that at longer intervals post-IR (3 h-24 h), proliferating myoblasts repair radiation-induced DNA damage less efficiently than quiescent satellite cells, however both repair more efficiently than differentiated cells *in vitro*, confirming results in isolated cells in culture. Although satellite cells maintained a high efficiency of repair either as isolated cells or in their niche, differences appeared more dramatic in myofibers and myoblasts compared to their *in vitro* counterparts.

These results were confirmed in fibers and myoblasts isolated from mice irradiated *in vivo* (Supplementary Fig. S4), including after multiple irradiation (Supplementary Fig. S5).

Although in all tested conditions the reduction of DSB-related markers post-IR was more efficient in satellite cells than in myoblasts and in differentiated cells, a few differences were noted. Myoblasts displayed similar repair kinetics under all tested conditions (middle panel, Supplementary Fig. S6). Conversely, satellite cells in fibers appeared to repair more efficiently than isolated satellite cells at least in the first 6 h post-IR, although they retained slightly higher levels of residual damage at 24 h post-IR (left panel). Differentiated cells displayed higher numbers of residual DSBs within the myofiber than in culture (right panel). In all cases, the kinetics of DSB-related foci was not significantly altered whether irradiation and repair took place *in vivo* or *in vitro*. Thus, the niche appears to have a minor impact on the repair of IR-induced DNA damage in satellite cells.

Efficient and accurate repair of DSB substrates by NHEJ in satellite cells

Reporter plasmids are then used to assay NHEJ in living cells (Sotiropoulou et al., 2010). Recently, we developed a method to assess the frequency of NHEJ using a highly sensitive plasmid-based assay that correlates robustly with repair on the chromosome, and allows enumerating the efficiency of DSB repair (Fig. 6A) (Chayot et al., 2010b, 2012). Fig. 6B shows that the repair efficiency was at least as high or higher in satellite cells ($f = 10^{-3}$) compared to MEFs ($f = 5 \times 10^{-5}$; as in a previous report (Chayot et al., 2012)). Importantly, a 90% reduction of NHEJ was observed in satellite cells in the presence of a specific inhibitor of DNA-PKcs (NU7441 (Leahy et al., 2004)), which is a major mediator of NHEJ, compared to the controls (Fig. 6C), indicating that DNA-PKcs plays a crucial role in the repair of NHEJ substrates in satellite cells.

Sequencing and restriction digestion identified accurate repair events (Supplementary Fig. S7). We observed that satellite cells exhibited a strikingly more accurate repair of DSBs compared to MEFs ($4.8 \pm 0.2 \times 10^{-4}$, and $2.2 \pm 0.4 \times 10^{-5}$, respectively; the latter confirming previous findings (Chayot et al., 2012)) (Fig. 6D). Sequencing also revealed that inaccurate events consisted of either large deletions (>30 bp) or micromodifications (1–10 nucleotides) in similar proportions (not shown). Most deletions rely on microhomology (1–6 nucleotides, a hallmark of alternative-NHEJ compared to the

Figure 6 Assessment of the efficiency and accuracy of NHEJ in muscle stem cells. A) Satellite cells (FACS, *Tg:Pax7-nGFP* mice) were compared with MEFs after transfection with linearized or circular plasmid to calculate NHEJ efficiency, after plating for ampicillin (Amp) resistance (R) and chloramphenicol (Cm) sensitivity (S) (see Supplementary material); B) box plot (min to max) of NHEJ frequency in satellite cells (n = 6 mice), and MEFs (n = 3 independent cell cultures); C) satellite cells (n = 3 mice) and MEFs (n = 3 experiments) were treated with DMSO or DNA-PK inhibitor NU7441 dissolved in DMSO; percentage inhibition of NHEJ (mean ± SD) calculated compared to control. NHEJ inhibition observed with MEFs is in agreement with previous data (Chayot et al., 2012). D) Accurate repair events assayed by digestion of recovered plasmid with *EcoRV* (reconstitution of intact *EcoRV* site, see Fig. S6). Sequencing of several junctions confirmed accurate repair (not shown). Frequency of accurate events and events with micromodifications (±10 nucleotides) or detectable change in size of plasmid substrate (deletions) are shown. Repair events: n = 144 for satellite cells and n = 26 for MEFs. More than 96% of junctions in satellite cells were accurate; ~46% were accurate in MEFs. Mean ± SEM, unpaired t test, ***$p \leq 0.001$. Mean ± SD. E) Frequency of repair events in the presence of inhibitor NU7441. F) Sequences of clonal repair junctions (inaccurate events); an accurate repair event indicated on top. Columns 5′Δ and 3′Δ indicate number of deleted nucleotides; six nucleotides at junction site, shown for deletions > 23 nt. Microhomology is underlined in one DNA end, and size indicated in column MH. Nd = not determined. G) Scheme of prevalent types of junction reconstructed from sequencing clonal repair events. Top, blunt ends after EcoRV digestion and removal of intervening cassette. Single bases, vertical bars; with bases close to DNA termini in red (top strand) and blue (bottom strand). Pattern "a", accurate repair, ligation without processing of DNA ends. Pattern "b", inaccurate event (resection of two bases at each 3′ end; synapsis with 2 nt microhomology) generating a micro-modification. Pattern "c", deletion of bases on both strands at each DNA termini; synapsis through microhomology (1–6 bp observed, 1 bp shown).

more accurate classical-NHEJ) (Nussenzweig and Nussenzweig, 2007) (Figs. 6F–G). Notably, in satellite cells accurate events depend on classical NHEJ, since the frequency of these events dropped to 10% in the presence of the NHEJ inhibitor, whereas inaccurate events were minimally affected (micromodifications) or unaffected (deletions) (Fig. 6E). In contrast, in MEFs, accurate and inaccurate repair events were reduced in the presence of the NHEJ inhibitor. Compared to satellite cells and MEFs, myoblasts displayed highly variable repair efficiencies and accuracies, probably reflecting a larger sensitivity to experimental conditions and heterogeneities reflecting different levels of commitment, and they repaired either accurately or through large or small deletions, in the presence and in the absence of microhomology (not shown). These results indicate that the repair of NHEJ-substrates is not only more efficient, but also more accurate in satellite cells compared to fibroblasts, and that accurate events essentially depend on classical NHEJ, where DNA-PKcs plays a major role.

Discussion

Genotoxic stress and DNA damage are considered to be major causes of aging in mammals. The risk of mutation load and loss of genome integrity in adult stem cells has been proposed to be the principal cause of age related decline in tissue function (Ruzankina et al., 2008). Here we show that muscle stem cells repair DSBs significantly more efficiently than their committed progeny. Satellite cells are quiescent, and they repair much more efficiently than post-mitotic differentiated myonuclei, raising the question whether reversible and irreversible cell cycle exits affect repair efficiency. Interestingly, the stem cells retain this capacity independent of the niche. Although the repair efficiency varies slightly in different experimental conditions, satellite cells systematically repair IR-induced DNA damage more efficiently than their progeny under similar conditions. In addition, we show that NHEJ is a major mediator of this mechanism and that it operates with unexpected accuracy on DSB substrates. Notably, the efficiency of repair decreases as a function of cell differentiation, whereas the proliferation state *per se* is not a major determinant. These findings provide important information on how stem cells maintain genome integrity, and they could be extended to other systems, including cancer stem cells.

The fate of muscle stem cells in response to IR-induced genotoxic stress

High doses of limb irradiation, 18–25 Gy (Boldrin et al., 2012; Gross and Morgan, 1999; Heslop et al., 2000), result in the loss of the majority of muscle stem cells, and failure of the remaining cells to undergo mitosis resulting in the loss of regenerative capacity. In agreement with these findings, we showed that a fractionated 15 Gy total body irradiation regime reduced the stem cell numbers after 22 days. Lower IR doses (5 Gy; fractionated 6 Gy), at shorter times post-IR, did not result in depletion of satellite cells. Interestingly, up to 15% of stem cell progeny undergo cellular senescence in culture post-IR. It is not clear if this is also the case *in vivo* where cell clearing mechanisms, such as macrophage-dependent phagocytosis, could limit this analysis. Thus, even the highest doses of IR tested here were not sufficient to significantly compromise the function of the surviving muscle stem cells, in agreement with the cited reports.

Muscle stem cells display robust survival to 20 Gy of total body irradiation where about 90% resist to apoptosis while up to two thirds of cells in the total muscle tissue undergo apoptosis. Further, less than 70% of differentiated MEFs survive after 5 Gy IR (Chayot et al., 2010a). These data are compatible with residual muscle regeneration observed after 18 Gy irradiation (Boldrin et al., 2012). The striking resistance of muscle stem cells to apoptosis following DNA damage is compatible with the observed high expression levels of antiapoptotic *Bcl2* compared to myoblasts and differentiated cells. This was also the case for Bcl2-dependent antiapoptotic response of hair-follicle-bulge stem cells compared to non-bulge cells (Sotiropoulou et al., 2010), whereas *Bcl2* is not expressed at higher levels in HSPCs compared to myeloid progenitors (Mohrin et al., 2010). Notably the expression of *p53* and of p53-target genes (including proapoptotic *Bax*) does not increase after irradiation of muscle stem cells. These observations suggest that in the presence of high levels of p53 and apoptotic genes, the anti-apoptotic response may be mediated by robust expression of *Bcl-2*. These findings also reveal that the relative resistance to apoptosis after DNA damage is differently regulated by members of the Bcl2 family in distinct types of stem cells.

Highly accurate repair of DSB substrates in stem cells

Two factors could seriously affect the fate of irradiated cells: persistence of unrepaired DNA damage and inaccuracy of repair. Unrepaired DNA damage post-IR is a potential threat for cycling cells where checkpoints arrest the cell cycle until repair is done, or cells undergo apoptosis. Persistent DNA damage can also trigger inaccurate repair, as is the case in NHEJ mutants (Nussenzweig and Nussenzweig, 2007). We observed that not only quiescent muscle stem cells but also proliferating myoblasts retain a low number of persistent 53BP1 foci/cell at 24 h post-IR, in all tested conditions. These findings indicate that myoblasts are able to recover within hours, whereas differentiated myonuclei are not. Nevertheless, proliferating muscle stem cells do not display a better repair efficiency than satellite cells, in spite of the expression of classical-NHEJ genes at least as much as in satellite cells, and higher levels of Rad51, a key effector of HR. Conversely, mobilized and pre-cultured HSPCs were reported to retain a lower number of persistent 53BP1 foci than quiescent HSPCs, compatible with the activation of recombination-dependent repair (Mohrin et al., 2010).

In the absence of irradiation, proliferating myoblasts were reported to display up to 2-fold more γH2AX-positive cells than quiescent muscle stem cells (Cousin et al., 2013). This labeling is compatible with replication-induced DNA damage (Branzei and Foiani, 2010), for 53BP1 labeling (Lukas et al., 2011), and for genome-wide γH2AX enrichment in cycling *versus* resting cells in the absence of IR (Seo et al., 2012). Therefore, proliferating muscle stem cells undergo a

larger number of endogenous DNA damage events compared to quiescent cells, as expected for cycling cells, rather than showing selective accumulation of DNA damage.

A major finding from the present study is that muscle stem cells repair DSBs with high efficiency and accuracy. The accurate repair of these substrates in the stem cells is dependent on the key classical-NHEJ factor DNA-PKcs, which promotes synapsis of the DNA ends and activates other components of the repair machinery (Dobbs et al., 2010). The inhibition of DNA-PKcs specifically drops accurate events by about 10-fold. This capacity of the stem cells to perform accurate repair differs markedly with that of HSPCs where significant genomic rearrangements were observed (Mohrin et al., 2010).

Radiation-resistance and DNA repair efficiency

Regeneration is a multifactorial process that involves multiple cell types and a functional niche (Boldrin et al., 2012; Saclier et al., 2013a,b; Yin et al., 2013). The niche might not have protective properties for high doses of strongly penetrating IR used here (Reisz et al., 2014), as it appears to be for endogenous oxidative stress (Pallafacchina et al., 2010). Interestingly, thrombopoietin, a factor of the hematopoietic niche, has been shown to stimulate DNA-PKcs-dependent repair in HSPCs (de Laval et al., 2013). The repair efficiency of muscle stem cells was reported to remain high, at least for replication-induced DSBs, in aged animals (Cousin et al., 2013), underscoring the robustness of DNA damage response in these cells. However, regeneration capacity has been reported to decline with age (Gopinath and Rando, 2008). In agreement with this notion, quiescent but not activated muscle stem cells from old mice display lower regeneration potential than those from young mice after high doses of irradiation (Cousin et al., 2013).

Colony formation of myogenic cells from mice with impaired DSB repair (SCID mice) was reported to be lower than in normal mice, suggesting that DNA damage repair plays a role in myoblast expansion in culture (Cousin et al., 2013). These authors also reported that in the absence of irradiation, regeneration efficiency was comparable in SCID and control mice, leading to the suggestion that DNA DSB repair deficiency does not impact regeneration capacity during aging. However, it is well established that the immune response, including infiltrate and macrophages, plays a critical role in the regeneration outcome (Saclier et al., 2013a). Further, the absence of T and B cells in SCID mice improves muscle regeneration due to diminution of pro-inflammatory macrophages (Farini et al., 2012), independently of DNA damage repair. Thus, the SCID model compromises the immune response and NHEJ, but not the homologous recombination. To resolve these issues, a model that affects only the DSB response, while maintaining the immune response, should be examined. This underscores the importance of initiating a systematic analysis of each cell type following irradiation induced DNA damage, as we have done for myogenic cells in the present study. Our data and recent findings on the accumulation of DNA damage-induced chromatin alterations in hair-follicle stem cells (Schuler and Rube, 2013) are compatible with the notion that DNA damage plays a role in stem cell aging.

How cell differentiation affects DSB repair remains an open question. It would be interesting to determine if depletion of the stem cell pool after irradiation is random, or whether subpopulations are more resistant (Kuang et al., 2007; Rocheteau et al., 2012; Shinin et al., 2006; Tajbakhsh, 2009) and if muscle stem cells use the same repair pathways compared to their committed progeny.

Nonstandard abbreviations

DDR	DNA damage repair
DMSO	dimethyl sulfoxide
DMEM	Dulbecco's modified Eagle medium
DSB	double-strand break
EDL	*extensor digitorum longus*
FCS	fetal calf serum
FACS	fluorescence activated cell sorting
GFP	green fluorescent protein
HR	homologous recombination
HSPCs	hematopoietic stem and progenitor cells
IR	ionizing radiation
MEFs	mouse embryonic fibroblasts
MPs	myeloid progenitors
NHEJ	non-homologous end-joining
PBS	phosphate buffered saline
PFA	paraformaldheyde
PI	propidium iodide
RT-qPCR	real time quantitive PCR
SCID	severe combined immunodeficiency
TA	*Tibialis anterior*

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2014.08.005.

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