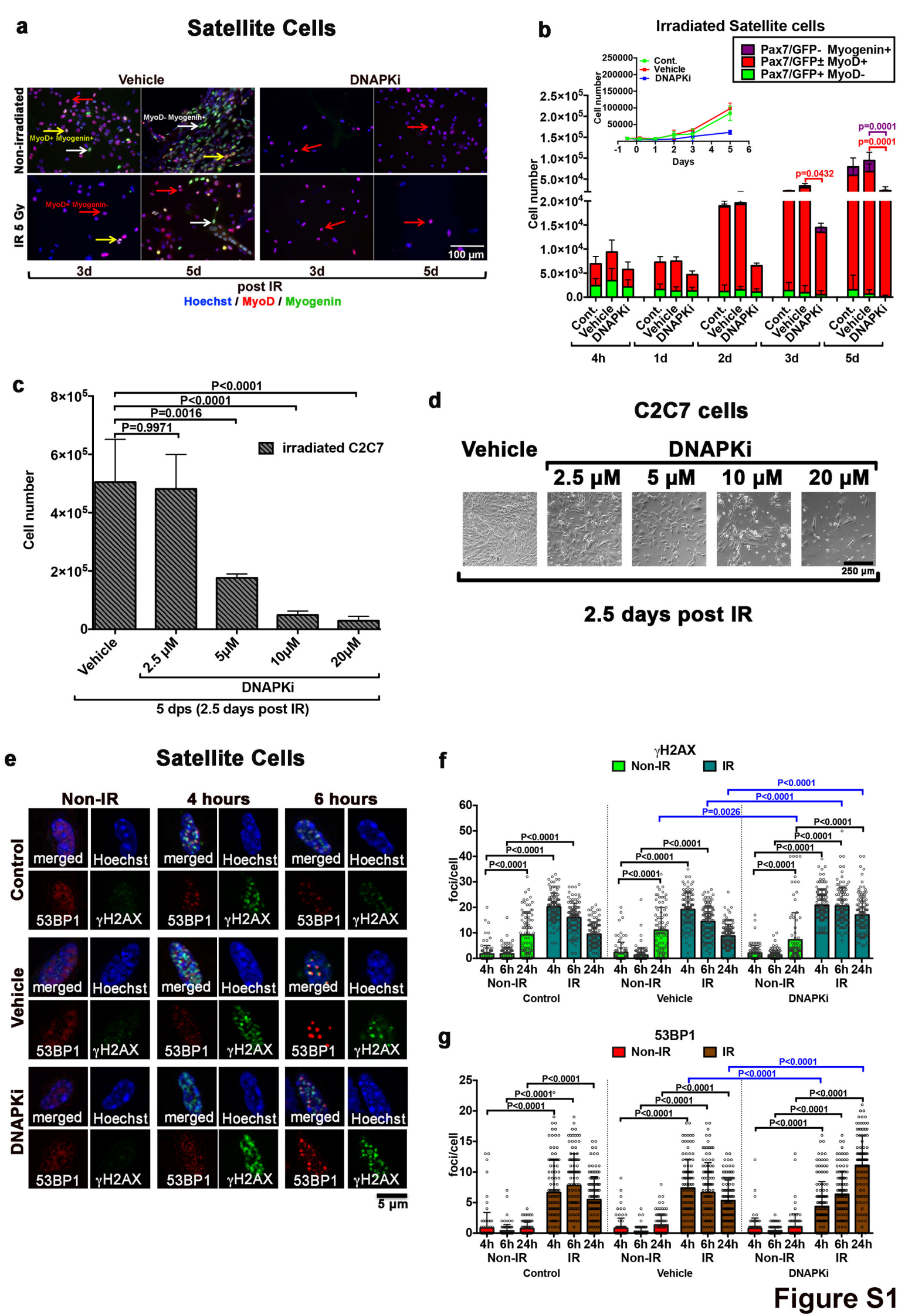
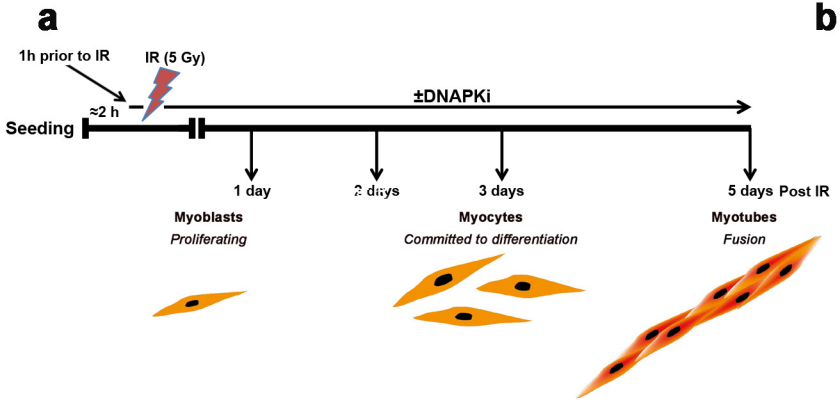
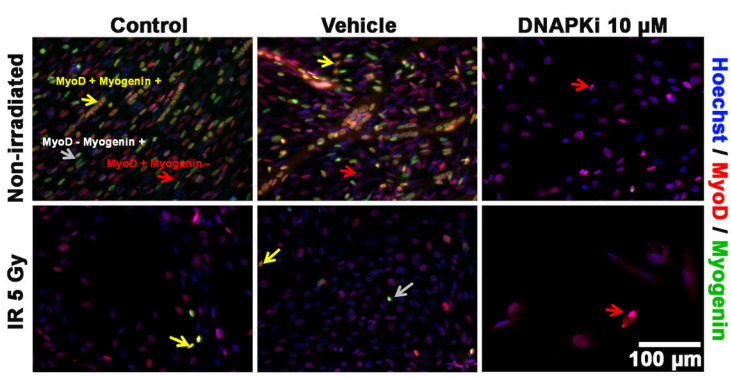
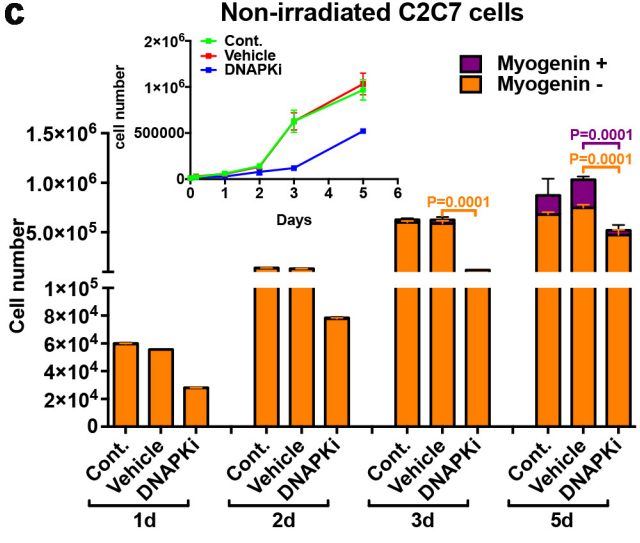

a
Satellite Cells
Vehicle
DNAPKi
Non-irradiated
IR 5 Gy
MyoD- Myogenin+
MyoD+ Myogenin+
MyoD+ Myogenin-
100 µm
3d
5d
3d
5d
post IR
Hoechst / MyoD / Myogenin
b
Irradiated Satellite cells
Pax7/GFP- Myogenin+
Pax7/GFP± MyoD+
Pax7/GFP+ MyoD-
Cont.
Vehicle
DNAPKi
Cell number
Days
p=0.0001
p=0.0001
p=0.0432
Cont.
Vehicle
DNAPKi
4h
1d
2d
3d
5d
c
P<0.0001
P<0.0001
P=0.0016
P=0.9971
irradiated C2C7
Cell number
Vehicle
2.5 µM
5µM
10µM
20µM
DNAPKi
5 dps (2.5 days post IR)
d
C2C7 cells
Vehicle
DNAPKi
2.5 µM
5 µM
10 µM
20 µM
250 µm
2.5 days post IR
e
Satellite Cells
Non-IR
4 hours
6 hours
Control
Vehicle
DNAPKi
merged
Hoechst
53BP1
γH2AX
5 µm
f
γH2AX
Non-IR
IR
foci/cell
P<0.0001
P=0.0026
4h 6h 24h 4h 6h 24h
Non-IR IR
Control
Vehicle
DNAPKi
g
53BP1
Non-IR
IR
foci/cell
P<0.0001
P<0.0001°
Control
Vehicle
DNAPKi

Figure S1

C2C7 cells







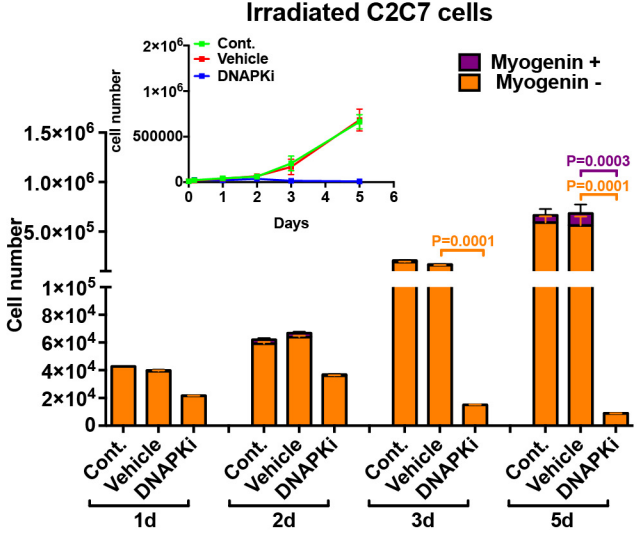


Figure S2

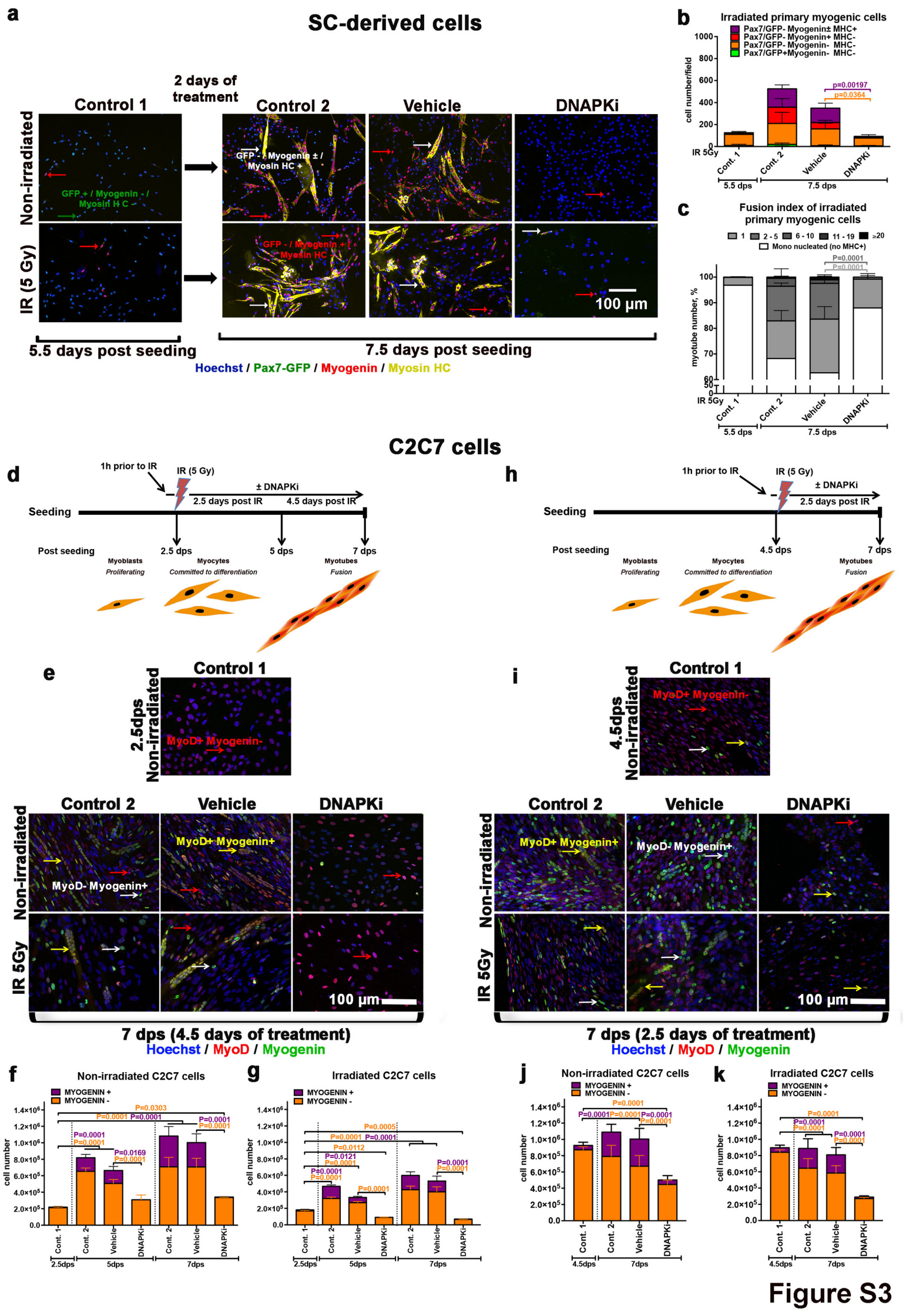

a
SC-derived cells
2 days of treatment
Control 1
Control 2
Vehicle
DNAPKi
Non-irradiated
IR (5 Gy)
GFP + / Myogenin - / Myosin H C -
GFP - / Myogenin ± / Myosin HC +
GFP - / Myogenin + / Myosin HC
100 µm
5.5 days post seeding
7.5 days post seeding
Hoechst / Pax7-GFP / Myogenin / Myosin HC
b
Irradiated primary myogenic cells
Pax7/GFP- Myogenin± MHC+
Pax7/GFP- Myogenin+ MHC-
Pax7/GFP- Myogenin- MHC-
Pax7/GFP+Myogenin- MHC-
cell number/field
p=0.00197
p=0.0364
IR 5Gy
Cont. 1
Cont. 2
Vehicle
DNAPKi
5.5 dps
7.5 dps
c
Fusion index of irradiated primary myogenic cells
1
2 - 5
6 - 10
11 - 19
≥20
Mono nucleated (no MHC+)
P=0.0001
P=0.0001
myotube number, %
C2C7 cells
d
1h prior to IR
IR (5 Gy)
± DNAPKi
2.5 days post IR
4.5 days post IR
Seeding
Post seeding
2.5 dps
5 dps
7 dps
Myoblasts
Proliferating
Myocytes
Committed to differentiation
Myotubes
Fusion
h
1h prior to IR
IR (5 Gy)
± DNAPKi
2.5 days post IR
Seeding
Post seeding
4.5 dps
7 dps
Myoblasts
Proliferating
Myocytes
Committed to differentiation
Myotubes
Fusion
e
Control 1
2.5dps Non-irradiated
MyoD+ Myogenin-
i
Control 1
4.5dps Non-irradiated
MyoD+ Myogenin-
Control 2
Vehicle
DNAPKi
Non-irradiated
IR 5Gy
MyoD- Myogenin+
MyoD+ Myogenin+
100 µm
7 dps (4.5 days of treatment)
Hoechst / MyoD / Myogenin
Control 2
Vehicle
DNAPKi
Non-irradiated
IR 5Gy
MyoD+ Myogenin+
MyoD- Myogenin+
100 µm
7 dps (2.5 days of treatment)
Hoechst / MyoD / Myogenin
f
Non-irradiated C2C7 cells
MYOGENIN +
MYOGENIN -
cell number
P=0.0303
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0169
P=0.0001
P=0.0001
P=0.0001
Cont. 1
Cont. 2
Vehicle
DNAPKi
2.5dps
5dps
7dps
g
Irradiated C2C7 cells
MYOGENIN +
MYOGENIN -
cell number
P=0.0005
P=0.0001
P=0.0001
P=0.0112
P=0.0121
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0001
Cont. 1
Cont. 2
Vehicle
DNAPKi
2.5dps
5dps
7dps
j
Non-irradiated C2C7 cells
MYOGENIN +
MYOGENIN -
cell number
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0001
Cont. 1
Cont. 2
Vehicle
DNAPKi
4.5dps
7dps
k
Irradiated C2C7 cells
MYOGENIN +
MYOGENIN -
cell number
P=0.0001
P=0.0001
P=0.0001
P=0.0001
P=0.0001
Cont. 1
Cont. 2
Vehicle
DNAPKi
4.5dps
7dps

Figure S3

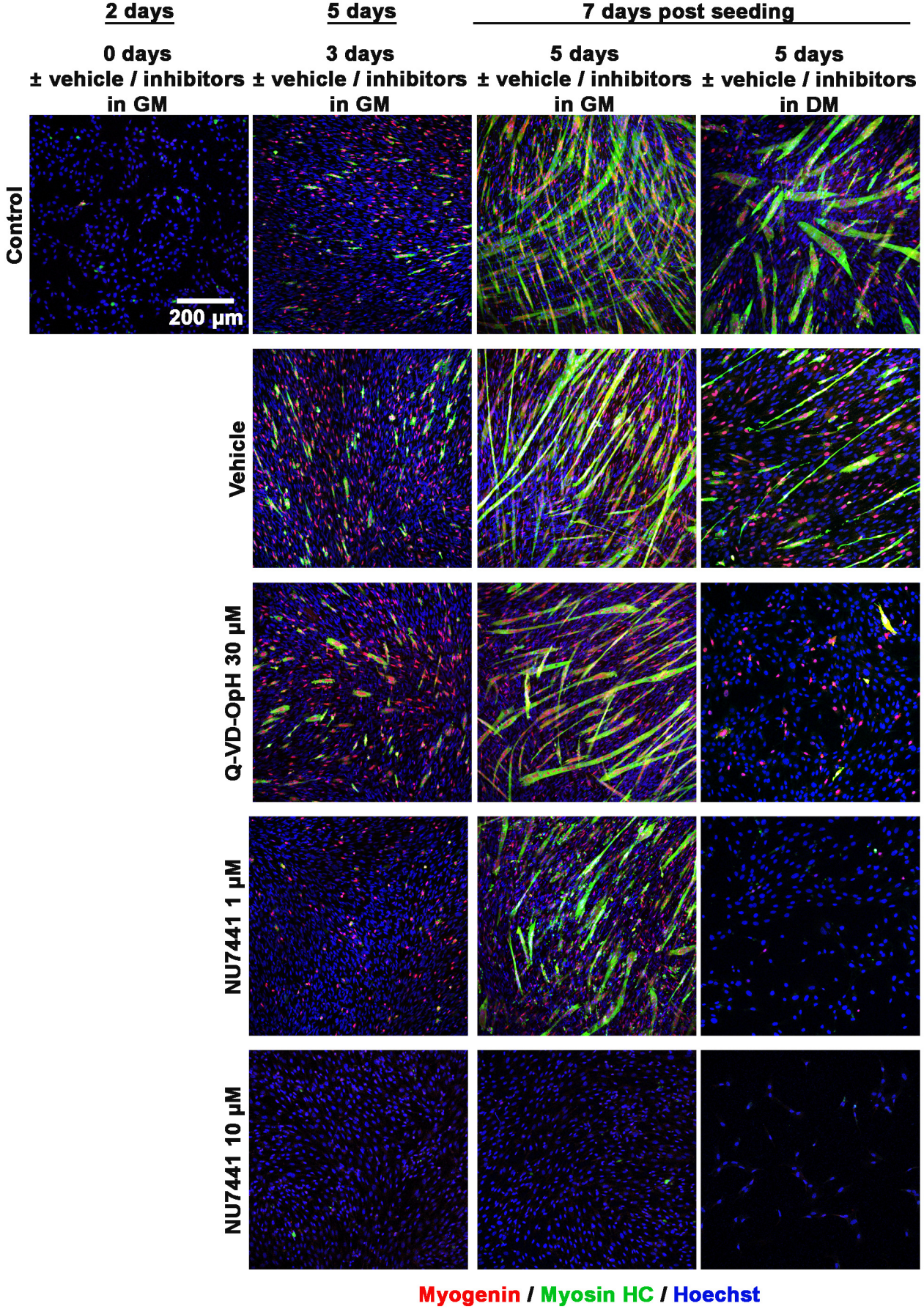
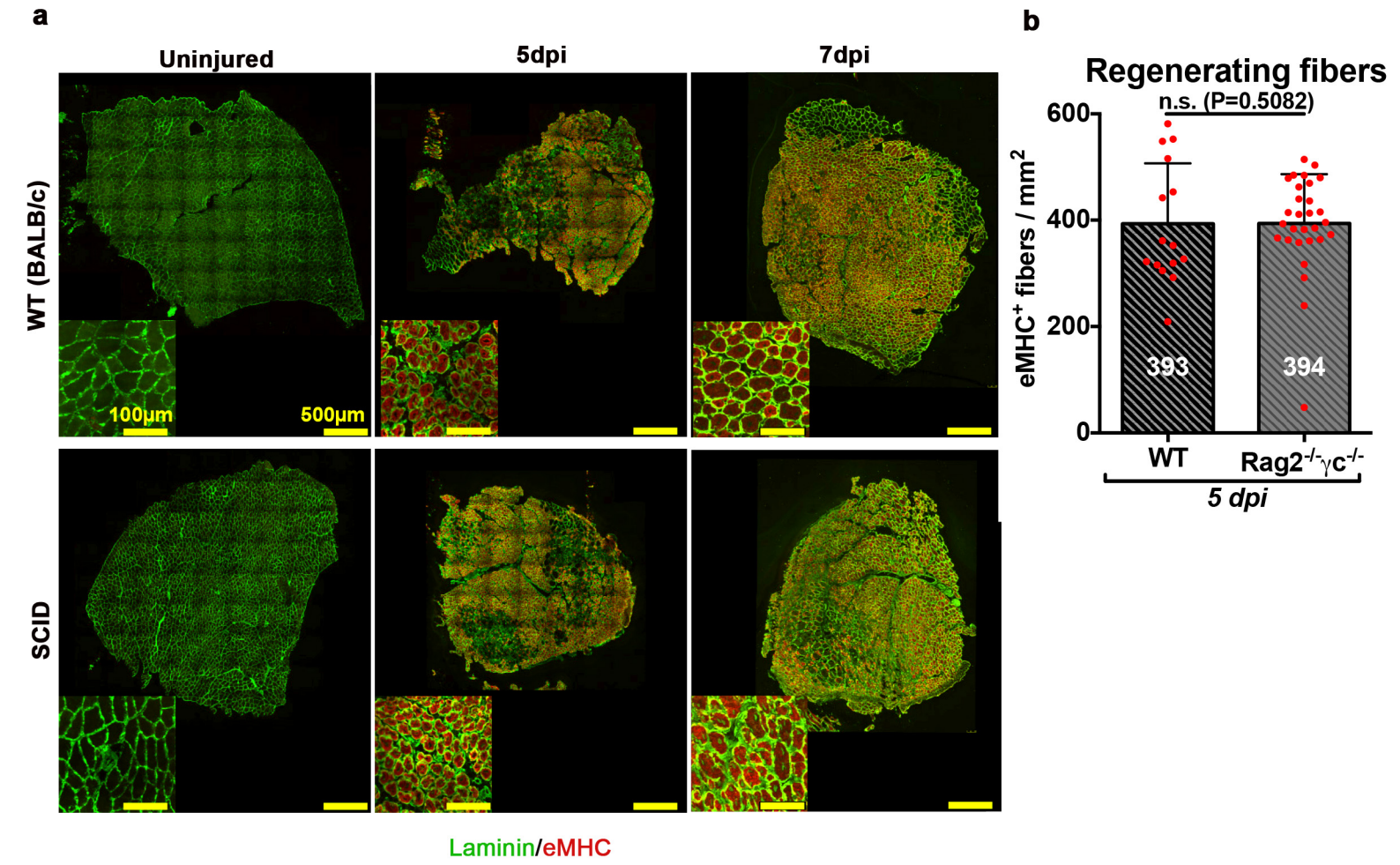

2 days
5 days
7 days post seeding
0 days
± vehicle / inhibitors
in GM
3 days
± vehicle / inhibitors
in GM
5 days
± vehicle / inhibitors
in GM
5 days
± vehicle / inhibitors
in DM
Control
200 µm
Vehicle
Q-VD-OpH 30 µM
NU7441 1 µM
NU7441 10 µM
Myogenin / Myosin HC / Hoechst

Figure S4


a
Uninjured
5dpi
7dpi
WT (BALB/c)
SCID
100µm
500µm
Laminin/eMHC
b
Regenerating fibers
n.s. (P=0.5082)
eMHC+ fibers / mm2
600
400
200
0
393
394
WT
Rag2-/-γc-/-
5 dpi

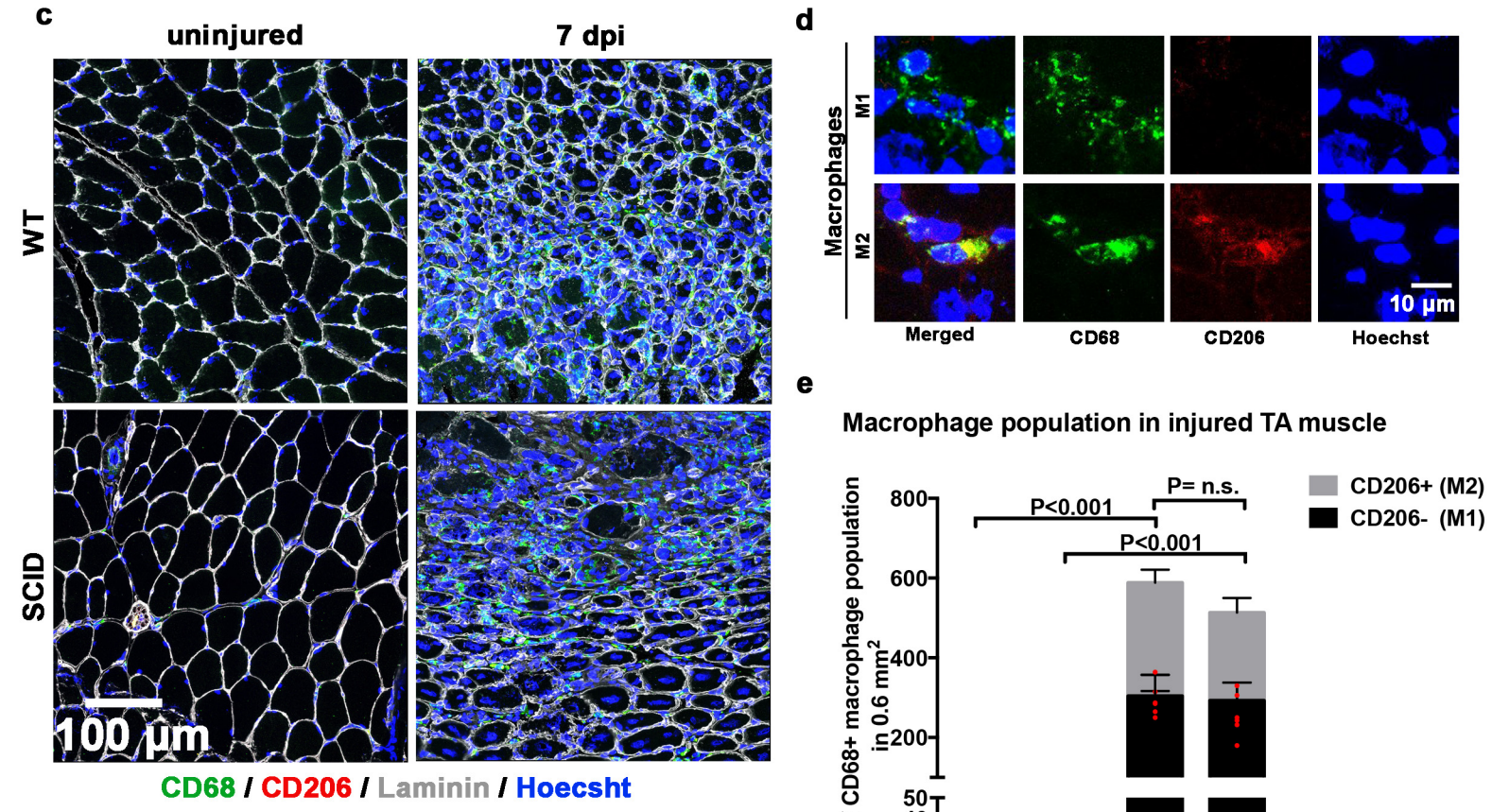

c
uninjured
7 dpi
WT
SCID
100 µm
CD68 / CD206 / Laminin / Hoecsht
d
Macrophages
M1
M2
Merged
CD68
CD206
Hoechst
10 µm
e
Macrophage population in injured TA muscle
CD206+ (M2)
CD206- (M1)
P<0.001
P= n.s.
P<0.001
number of CD68+ macrophage population in 0.6 mm2
800
600
400
200
50
40
30
20
10
0
WT
SCID
WT 7dpi
SCID 7 dpi

Figure S5

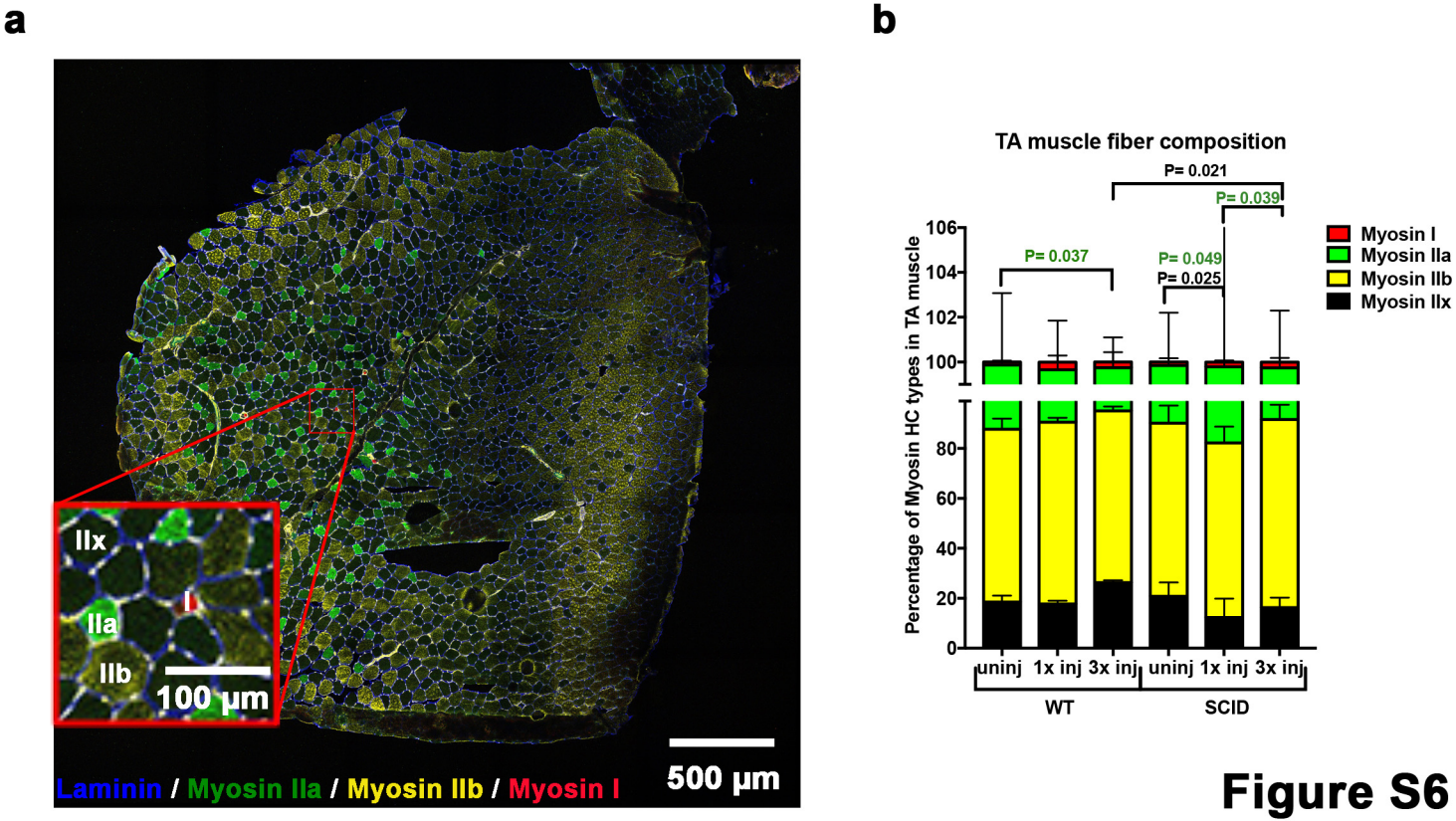

a
b
IIx
I
IIa
IIb
100 µm
500 µm
Laminin / Myosin IIa / Myosin IIb / Myosin I
TA muscle fiber composition
P= 0.021
P= 0.039
P= 0.037
P= 0.049
P= 0.025
Myosin I
Myosin IIa
Myosin IIb
Myosin IIx
Percentage of Myosin HC types in TA muscle
106
104
102
100
80
60
40
20
0
uninj
1x inj
3x inj
uninj
1x inj
3x inj
WT
SCID

Figure S6

Supplementary Tables

Weight of TA muscle (mg)					
No Injury					
11 weeks old	15 weeks old	19 weeks old	11 weeks old	15 weeks old	19 weeks old
WT	**WT**	**WT**	**SCID**	**SCID**	**SCID**
58.4	56.2	60.9	57.8	62.0	66.4
78.3	65.0	58.7	57.3	60.9	57.3
69.4	65.0	62.4	68.9	58.3	55.1
61.6	68.0	68.4	58.5	51.2	54.4
58.1	NA	NA	49.7	40.0	58.8
1 x injury	2 x injuries	3 x injuries	1 x injury	2 x injuries	3 x injuries
11 weeks old	15 weeks old	19 weeks old	11 weeks old	15 weeks old	19 weeks old
WT	**WT**	**WT**	**SCID**	**SCID**	**SCID**
63.3	77.8	86.5	57.1	75.5	78.3
83.1	80.9	88.1	54.5	76.0	66.4
72.3	86.3	85.7	66.5	71.6	65.1
47.0	73.8	80.8	60.4	62.0	67.3
67.5	NA	NA	61.3	68.6	69.5

Supplementary Table S1: TA muscle weight after 1, 2, or 3 injuries, or uninjured, from WT and SCID mice. NA: not applicable.

Genotype	Phenotype	Genetic Background	References
Pax7$^{nGFP/+}$	Pax7 – GFP positive	B6D2/F1	[1]
CB17/lcr-*Prkdc*$^{scid/scid}$/lcrlcoCrl	DNAPK deficient, B- and T-cell deficient	Balb/c	[2,3]
Balb/cAnN $^{+/+}$	WT control for SCID	Balb/c	-
Rag2$^{-/-}$ *γc*$^{-/-}$	Rag2 deficient, B-, T-cells and NK deficient	C57BL/B6	[4]
B6 $^{+/+}$	WT control for Rag2$^{-/-}$ γc$^{-/-}$	C57BL/B6	-

Supplementary Table S2: List of mice strains used. The genotype, phenotype, and genetic background of each strain are indicated.

Mission shRNA Library (Sigma)	Plasmid	Target gene
Ref:SHC002	pLKO.1_Puro control	shRNA (sh Control) (SCR)
Ref:TRCN0000023863	pLKO.1_Puro DNAPKcs	shRNA (sh DNAPKcs-1)
Ref:TRCN0000361373	pLKO.1_Puro DNAPKcs	shRNA (sh DNAPKcs-2)
Ref:TRCN0000310882	pLKO.1_Puro Akt2	shRNA (sh Akt2-1)
Ref:TRCN0000301381	pLKO.1_Puro Akt2	shRNA (sh Akt2-2)
Ref:TRCN0000304684	pLKO.1_Puro Akt	shRNA (sh Akt1)

Supplementary Table S3: List of shRNAs. Reference numbers, plasmid codes, and target genes are indicated. SCR, scramble.

Regions	Primer	Sequence (5′-3′)	Reference
MyoG-1	Forward	GTTGAGGCTGCCAAGAAGAC	This study
	Reverse	GTCTCCCTTCCTTCCTGGTC	
MyoG-2	Forward	TTTGTCCCAACCAGGAAGAG	This study
	Reverse	CTGGAGGCAGAAGAAGTTGG	
MyoG-4	Forward	TGTAGCTCTGATGGCCTGTG	This study
	Reverse	TCCCTCCCCTTTTGTTTTCT	
MyoG-5	Forward	GCTGACAGAGGCCAAGATTC	This study
	Reverse	TGCTTTCCCCTCAATAATGC	
MyoG-6	Forward	GGAATCACATGTAATCCACTGG	[5]; this study
	Reverse	TCACACCAACTGCTGGGT	

Supplementary Table S4: Primer pairs used to assess the promoter region of *MyoG*. The primers have been designed to amplify regions up to 7kbp upstream of the *MyoG* initiation of transcription, where an enhancer region has been previously described [6].

References Supplementary Material

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2. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. *Nature* 1983, **301**(5900)**:** 527-530.
3. Bosma MJ, Carroll AM. The SCID mouse mutant: definition, characterization, and potential uses. *Annual review of immunology* 1991, **9:** 323-350. DOI: 10.1146/annurev.iy.09.040191.001543.
4. Colucci F, Soudais C, Rosmaraki E, Vanes L, Tybulewicz VL, Di Santo JP. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. *Journal of immunology (Baltimore, Md : 1950)* 1999, **162**(5)**:** 2761-2765.
5. Londhe P, Davie JK. Sequential association of myogenic regulatory factors and E proteins at muscle-specific genes. *Skeletal muscle* 2011, **1**(1)**:** 14. DOI: 10.1186/2044-5040-1-14.
6. Faralli H, Dilworth FJ. Turning on myogenin in muscle: a paradigm for understanding mechanisms of tissue-specific gene expression. *Comparative and functional genomics* 2012, **2012:** 836374. DOI: 10.1155/2012/836374.

Supplementary Figure Legends

Supplementary Figure S1. **Inhibition of DNA-PKcs blocks myogenic differentiation of satellite cells in the presence and in the absence of irradiation (induced DSBs)**

a) Representative merged images of immunofluorescent labelling of cells 5 days post-IR (and non-irradiated controls): MyoD (red), Myogenin (green), and nuclei counterstained with Hoechst (blue). Representative cells with progressively increased levels of differentiations are indicated with red arrows ($MyoD^+/Myogenin^-$ cells, in red), yellow arrows ($MyoD^+/Myogenin^+$ cells, in yellow), and white arrows ($MyoD^-/Myogenin^+$cells, in green). **b**) Histograms of myogenic differentiation and growth curves of non-irradiated cells ± DNAPKi. Proliferation evaluated with the cell number, and differentiation with immunofluorescence of myogenic markers. Each condition was tested with SC derived from n=3-7 mice, mean ± SD. Myogenic markers have been analysed in 5-10 fields/condition (2,000-5,000 cells), and extrapolated to the total cell number. Significance evaluated by by 2-way ANOVA ($F=8.6$, $DFn=28$, $DFd=147$, $p<0.000001$), with post-hoc Dunnett's multiple comparisons test; significant P values are indicated in histogram. P values and bars and are of the same colour as the category that is compared. Panels a-b, experimental conditions as in Fig. 1a. **c**) C2C7 cell number upon irradiation in the presence of increasing doses of DNAPKi, or in the absence of inhibitor. Cells were treated and irradiated at 2.5 dps and harvested at 5 dps. N=3 independent experiments, mean ± SD, Significance by 2-way ANOVA ($F=6.20$, $DFn=4$, $DFd=22$, $p<0.0017$), with post-hoc Dunnett's multiple comparisons test against vehicle (DMSO), significant P values are indicated on histogram. The concentration of DNAPKi corresponding to the lowest dose that blocks cell proliferation upon irradiation (10 μM) was used in this study, unless otherwise specified. **d**) Representative images of panel c. **e)** Representative images of SCs cells 4h and 6h post-IR, and non-irradiated controls, immunostained for the DSB repair markers γH2AX (green) and 53BP1 (red), nuclei

counterstained with Hoechst (blue), and merge. Efficiency of DSB repair is evaluated by disappearance of repair markers. Histogram report the number of **f)** γH2AX foci/cell (nucleus) and **g)** 53BP1 foci/cell (nucleus) from 4h to 24h post irradiation. Mean ± SEM for each condition. Significance by 1-way ANOVA (γH2AX: F=174, DFn=17, DFd=1892, p<0.0001. 53BP1: F=103.2, DFn=17, DFd=1892, p<0.0001) significant P values by post-hoc Tukey's multiple comparisons test are indicated on histogram n=30-40 cells/condition. N= 3 independent experiments.

Supplementary Figure S2. **DNA-PKcs inhibitor blocks differentiation of C2C7 cells in the presence and in the absence of irradiation**

a) Schematic representation of the experiment. Immortalized myogenic C2C7 cells were pre-treated with DNA-PKi before irradiation (or no-IR) and kept in culture for 1 to 5 days before analysis. **b)** Representative merge images of immunofluorescent labelling of cells 5 days post-IR (and non-irradiated controls). Proliferation was evaluated by the cell number and differentiation by myogenic markers, as in Fig 1. MyoD (red), Myogenin (green), and nuclei are counterstained with Hoechst (blue). Representative cells with progressively increased stage of differentiations are indicated with red arrows ($MyoD^+/Myogenin^-$ cells, in red), yellow arrows ($MyoD^+/Myogenin^+$ cells, in yellow), and white arrows ($MyoD^-/Myogenin^+$cells, in green). Undifferentiated C2C7 myoblasts are originally $MyoD^+$. Histograms of myogenic differentiation and growth curves of **c)** non-irradiated and **c)** irradiated C2C7 cells ± DNAPKi. Significance by 2-way ANOVA (Non-irradiated: F=112.8, DFn=11, DFd=47, p<0.0001. Irradiated: F=87.06, DFn=11, DFd=48, p<0.0001), with post-hoc Dunnett's multiple comparisons test, significant P values are indicated on the histogram. The P values and error bars are of the same colour as the category that is compared. n=3

experiments per condition, mean ± SD. Myogenic markers have been analysed in 5-10 fields/condition (2,000-5,000 cells), and extrapolated to the total cell number.

Supplementary Figure S3. DNA-PKcs inhibitor affects myogenesis by reducing proliferation and blocking differentiation of myogenic cells

a) Immunolabelling of SC-derived cells at 5.5 dps without DNAPKi treatment (control 1; at this time point cells were essentially myoblasts [88% $Myog^-$ and Myosin Heavy Chain, MHC^-, a structural marker for myotube formation]) and 7.5 dps (with and without [control 2 and vehicle] DNAPKi treatment). Merge of MHC (yellow), Myogenin (red), Pax7-nGFP (green) and Hoechst counterstaining (nuclei, blue). Representative MHC^+ structures are indicated with a white arrow, $Myogenin^+$ cells (red) with a red arrow, and $Pax7\text{-}GFP^+$ cells (green) with a green arrow. **b)** Histograms display the cell number and differentiation stage of irradiated SC-derived cells treated or not with DNAPKi, and defined by the indicated combinations of myogenic markers (as in Fig. 1d). Myogenic markers have been analysed in 5-10 fields/condition and extrapolated to the total cell number. mean ± SD for each category. Significance by 2-way ANOVA ($F=2.53$, $DFn=9$, $DFd=32$, $p=0.0255$), with post-hoc Dunnett's multiple comparisons test; significant P values are indicated in histogram. P values and error bars are of the same colour as the category that is compared. **c)** Fusion indexes of irradiated SC-derived cells by enumeration of nuclei/myotube (as in Fig. 1e). Significance by 2-way ANOVA ($F=31.70$, $DFn=15$, $DFd=40$, $p<0.0001$), with post-hoc Dunnett's multiple comparisons test; significant P values are indicated in histogram. P values and error bars are of the same colour as the category that is compared. Significance bars are of the same colour as the category that is compared. n=3 experiments, n=2,500-5,000 cells analysed/condition, mean ± SD. Panels a-c, experimental conditions as in Fig. 1c. Treatment with DNAPKi in committed C2C7 cells (**d-g**) and during differentiation (**h-k**). For simplification,

differentiation was evaluated considering Myogenin$^+$ *versus* Myogenin$^-$ cells. **d**) Schematic representation of the experiment. At 2.5 dps, during mid myogenesis cells were pretreated for 1h with 10 µM of DNAPKi or the corresponding volume of vehicle before irradiation (or were not irradiated), and then analysed at 5 and 7 dps. This experiment mimics the condition of SCs in Fig. 1c, as indicated by comparable ratio of Myog$^+$/Myog$^-$ cells in control samples (see panel a). Proliferation evaluated with the cell number, and differentiation assessed with immunofluorescence of myogenic markers (MyoD, Myogenin). **e)** Representative merge images of immunofluorescent labelling of cells at 2.5dps and 7dps: MyoD (red), Myogenin (green), nuclei counterstained with Hoechst (blue). Representative cells with progressively increased levels of differentiations are indicated: red arrows (MyoD$^+$/Myogenin$^-$ cells, in red), yellow arrows (MyoD$^+$/Myogenin$^+$ cells, in yellow), and white arrows (MyoD$^-$ /Myogenin$^+$cells, in green). Histograms of the cell number and differentiation state of **f)** non-irradiated and **g)** irradiated C2C7 cells treated or not with DNAPKi. The differentiation state is defined by Myogenin immunolabelling: Myogenin$^-$ cells [undifferentiated] and Myogenin$^+$ [differentiated] cells are shown in the orange and purple part of columns, respectively. Significance by 2-way ANOVA (Non-irradiated: $F=7.997$, $DFn=6$, $DFd=107$, $p<0.0001$. Irradiated: $F=12.68$, $DFn=6$, $DFd=103$, $p<0.0001$), with post-hoc Dunnett's multiple comparisons test, significant P values are indicated in histogram. The P values and error bars are of the same colour as the category that is compared. **h)** Schematic representation of the experiment, which consists in a later stage, with myogenic cells having extensively proliferated, and which are expected to differentiate and form myotubes before treatment. At 4.5 dps, during advanced myogenesis, cells were pretreated for 1h with 10 µM of DNAPKi or the corresponding volume of vehicle before irradiation (or were not irradiated), and then analysed at 7 dps. **i)** Representative merge images of immunofluorescent labelling of cells at 4.5 and 7 dps: the indications are in same colour as in panel e. Histograms of the cell number

and differentiation stage of **j)** non-irradiated and **k)** irradiated C2C7 cells treated (or not) with DNAPKi, and defined as in panels c and d. Significance by 2-way ANOVA (Non-irradiated: F=41.38, DFn=3, DFd=98, p<0.0001. Irradiated: F=39.58, DFn=3, DFd=100, p<0.0001), with post-hoc Dunnett's multiple comparisons test, significant P values are indicated in histogram. The P values and error bars are of the same colour as the category that is compared. n=3 experiments. The Myogenin marker has been analysed analysed in 5-10 fields/condition corresponding to n=2,500-5,000 cells/condition, and extrapolated to the total cell number; mean ± SD.

Supplementary Figure S4. DNA-PKcs acts upstream of caspases in myogenic differentiation

Representative merge images of immunofluorescent labelling of C2C7 cells at 2, 5, and 7 dps: Myogenin (red), Myosin HC (green), nuclei counterstained with Hoechst (blue). The experimental planning was as in Figure S3d. Until 2 dps cells were cultured in growth medium, GM (*i.e.* serum-rich). The cells were then treated with vehicle, or 30 μM of Caspase inhibitor (Caspi, Q-VD-Oph), or 1 μM and 10 μM (optimal concentration in GM (see Fig. S1c)) of NU7441 (DNAPKi) in GM or in differentiation medium (DM) (*i.e.* serum-depleted) until 5 dps and 7 dps.

Supplementary Figure S5. Regeneration in WT and SCID mice

a) Representative images of un-injured and regenerating TA sections of WT or SCID mice 5 and 7 dpi. Extracellular matrices bordered by laminin (green) and regenerating fibers marked with eMHC (red), nuclei counter-stained with Hoechst; (quantifications are shown in Fig. 5e-g). **b)** Quantification of regenerating $eMHC^+$ fibers/mm^2 on TA sections in WT (C57BL/B6) and immunodeficent *Rag2*$^{-/-}\gamma c^{-/-}$ mice. n=3 mice, 10 sections/condition, mean ± SD.

Significant P values between conditions by Mann Whitney test are indicated on the histograms. **c)** Representative images of macrophages in un-injured and regenerating TA sections of WT or SCID mice 7 dpi. Extracellular matrices are bordered by laminin (white) and global macrophages are marked with the pan-macrophage marker CD68 (green), M2 macrophages with CD206 (red) and counterstained with Hoechst (blue, nuclei). **d)** Representative zoomed-in images of macrophage populations: M1 (CD68+ CD206-, green) and M2 (CD68+ CD206+, (combined green and red), counterstained with Hoechst (blue). **e)** Quantification of M1, pro-inflamatory macrophages (CD68+ CD206-) and M2, anti-inflamatory macrophages (CD68+ CD206+) in un-injured and regenerating TA sections of WT or SCID mice 7 dpi. n=3 mice, 3 regions of 0.6 mm^2/condition, mean ± SD. Significance by 2-way ANOVA (F=7.89, DFn=3, DFd=8, p=0.009), with post-hoc Tukey's multiple comparisons test, significant P values are indicated in the histogram.

Supplementary Figure S6. Fiber composition of the TA muscle

a) Representative image an un-injured TA section labeled for different myosin HC isoforms. Extracellular matrices are bordered by laminin (blue) and isoforms of Myosin HC are labeled with Myosin type I (red) for slow fibers, Myosin type IIa (green) for fast oxydative fibers, and Myosin type IIb (yellow) for fast glycolytic fibers; non-labelled fibers are considered Myosin type IIx (black) for fast glycolytic/oxidative intermediate fibers. **b)** Histogram of percentage myosin HC in un-injured and regenerating TA sections of WT or SCID mice at 29 days post-injury (1 injury), and 89 days post injury (3 injuries) (experimental scheme in Fig 5a). n=3 mice, (1380-4587 fibers/condition) mean ± SD. Significance by 2-way ANOVA (F=3.48, DFn=15, DFd=72, p=0.0002), with post-hoc Tukey's multiple comparisons test, significant P values are indicated in histogram. P values are of the same colour as the category that is compared.