

## Supplementary Information

Interplay among p21<sup>Waf1/Cip1</sup>, MUSASHI-1 and Krüppel-like factor 4 in activation of  
*Bmi1*-CreER reserve intestinal stem cells after gamma radiation-induced injury

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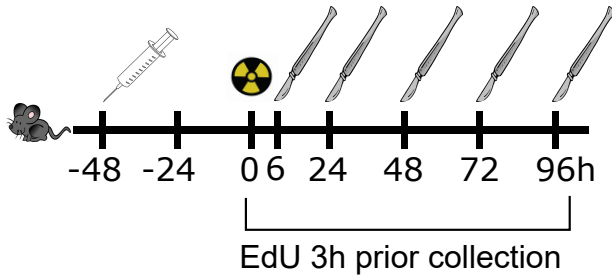
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**A**

Protocol 1

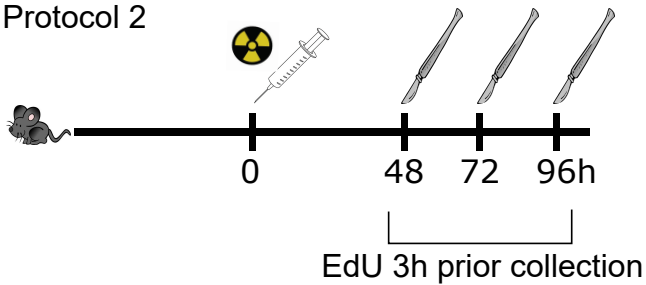


Tamoxifen

12 Gy TBI or sham

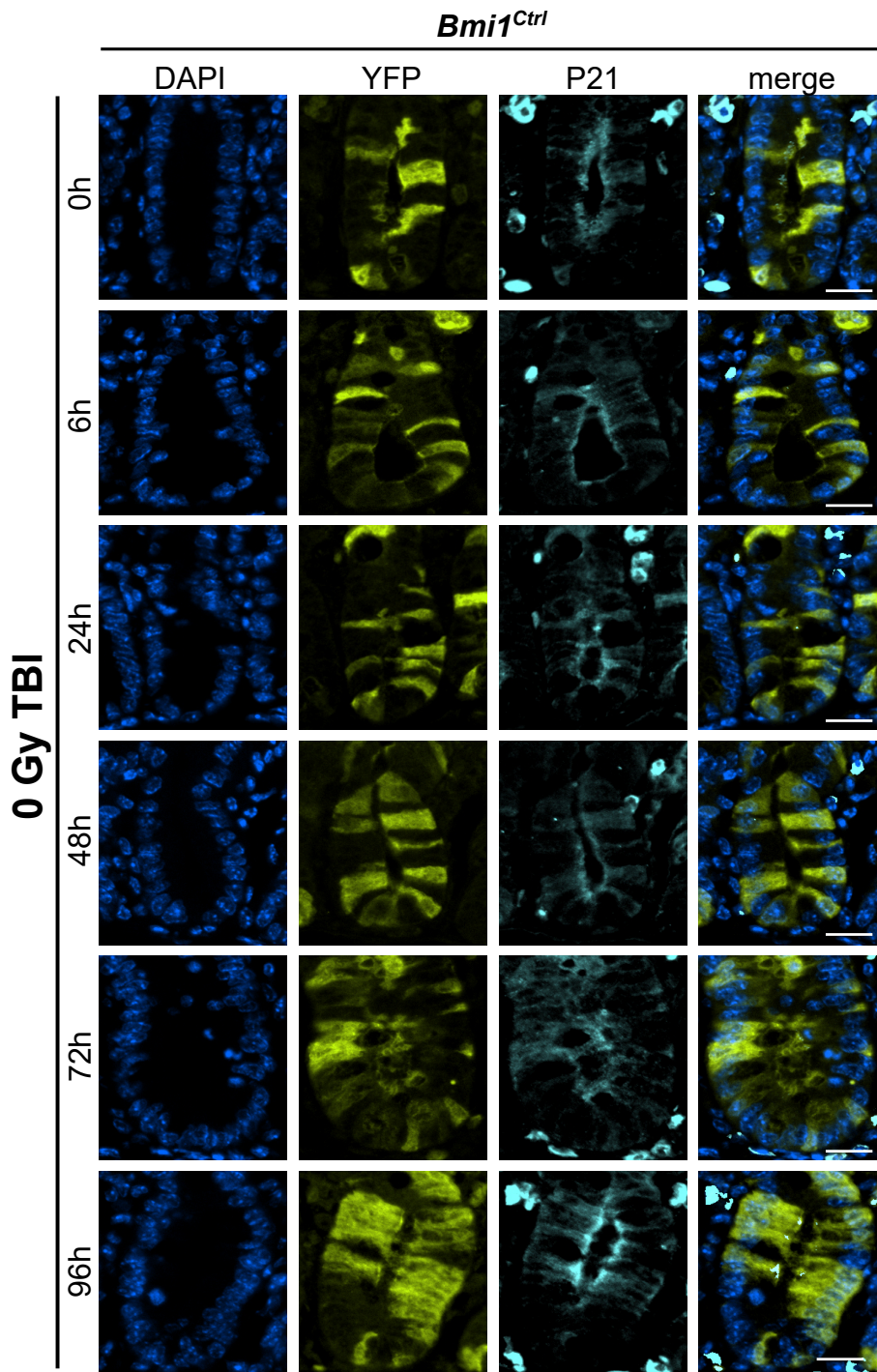
**B**

Protocol 2

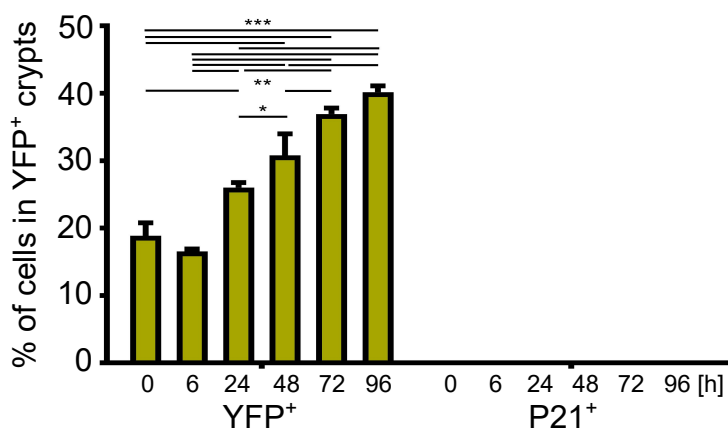


**Supplementary Figure 1.** Outline of the experimental design. (A) Eight-to 12-week-old female *Bmi1<sup>Ctrl</sup>* and *Bmi1<sup>ΔKlf4</sup>* mice were injected with tamoxifen 2 days prior treatment and sacrificed at 0, 6, 24, 48, 72 or 96 h after irradiation with total dose of 0 Gy (sham group) or 12 Gy (irradiated group) total body irradiation. Mice were injected with EdU 3 h prior to sacrifice. (B) Eight-to 12-week-old female *Bmi1<sup>Ctrl</sup>* and *Bmi1<sup>ΔKlf4</sup>* mice were injected with tamoxifen, irradiated at time 0 h with total dose of 0 Gy (sham group) or 12 Gy (irradiated group) total body irradiation and sacrificed at 48, 72 or 96 h after irradiation. Mice were injected with EdU 3 h prior to sacrifice

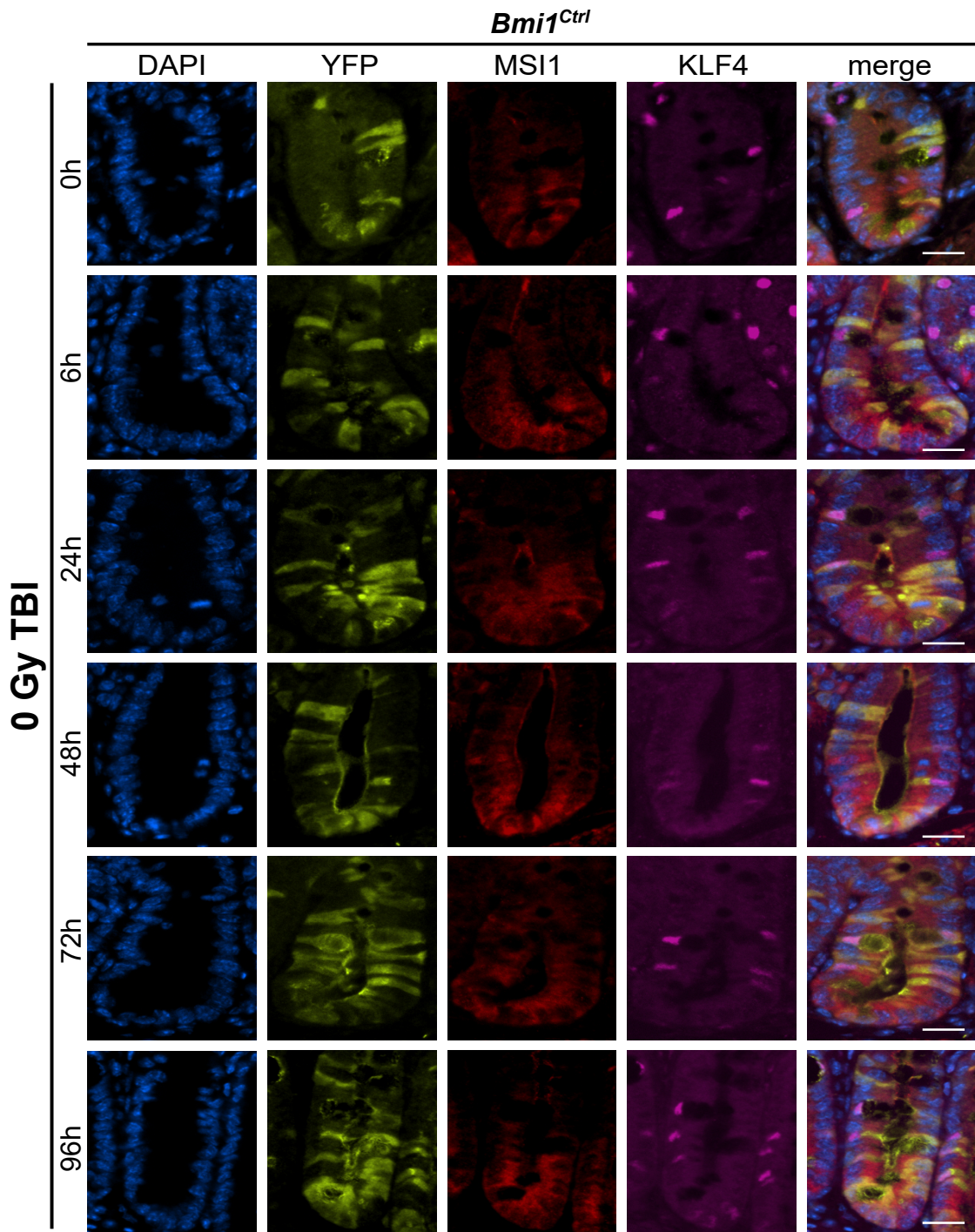
A



B

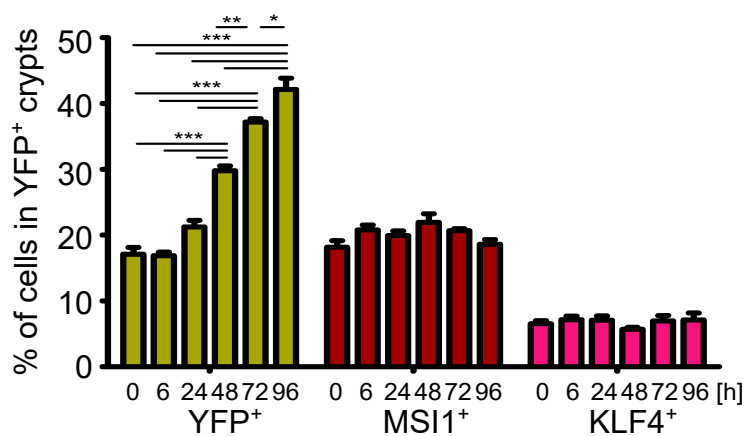


**Supplementary Figure 2.** Time-dependent p21<sup>Waf1/Cip1</sup> (P21) expression pattern in the YFP<sup>+</sup> crypts after 0 Gy TBI of the *Bmi1<sup>Ctrl</sup>* mice treated according to protocol 1 (Supplementary Fig. 1A). (A) Representative IF images of DAPI, YFP, and p21<sup>Waf1/Cip1</sup> staining in the PSI crypts at 0, 6, 24, 48, 72 and 96 h obtained under a fluorescence microscope. The scale bar represents 20  $\mu$ m. (B) Quantification of the percentage of YFP<sup>+</sup> or p21<sup>Waf1/Cip1</sup> cells in the YFP<sup>+</sup> crypts. Data are represented as the mean  $\pm$  SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 by one-way ANOVA.

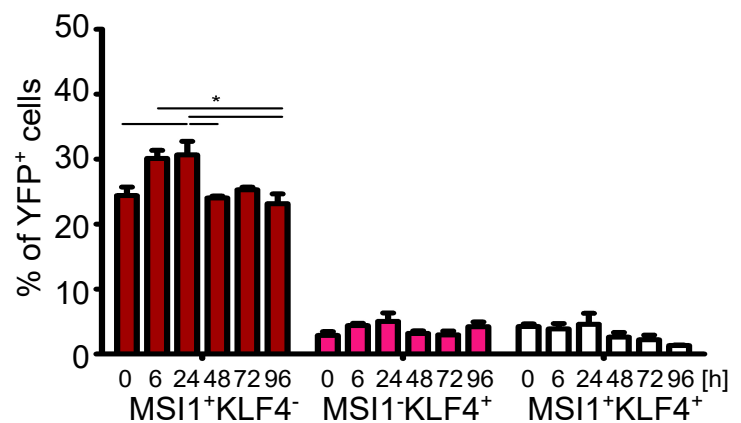


**Supplementary Figure 3.** Time-dependent MSI1 and KLF4 expression patterns in the YFP<sup>+</sup> crypts after 0 Gy TBI of the *Bmi1<sup>Ctrl</sup>* mice treated according to protocol 1 (Supplementary Fig. 1A). (A) Representative IF images of DAPI, YFP, MSI1 and KLF4 staining in the PSI crypts at 0, 6, 24, 48, 72 and 96 h obtained under a fluorescence microscope. The scale bar represents 20  $\mu$ m. (B) Quantification of the percentage of YFP<sup>+</sup> or MSI1<sup>+</sup> or KLF4<sup>+</sup> cells in the YFP<sup>+</sup> crypts. (C) Quantification of the percentage of YFP<sup>+</sup> cells co-stained with MSI1, KLF4 or MSI1 and KLF4 together. Data are represented as the mean  $\pm$  SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 by one-way ANOVA.

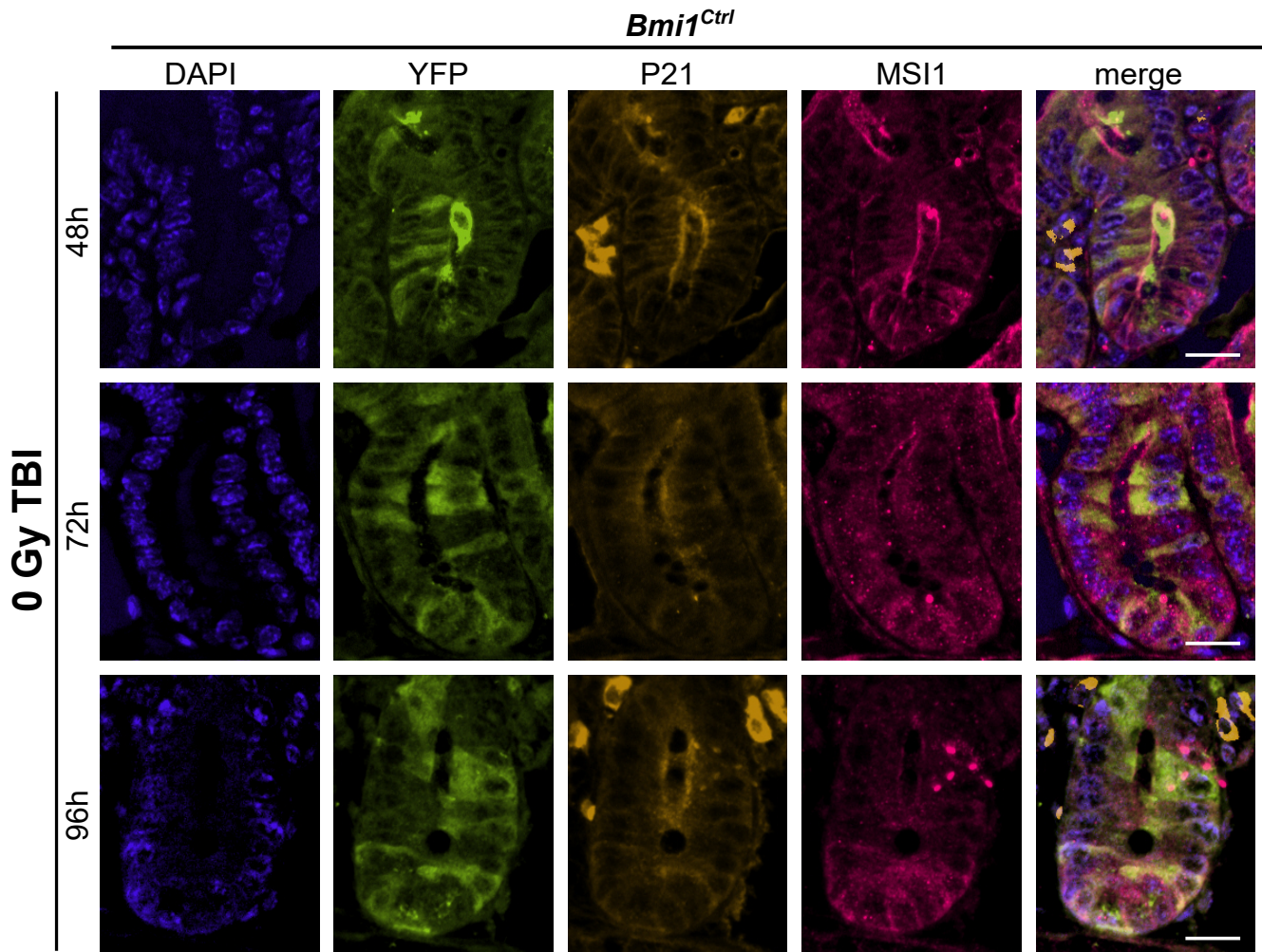
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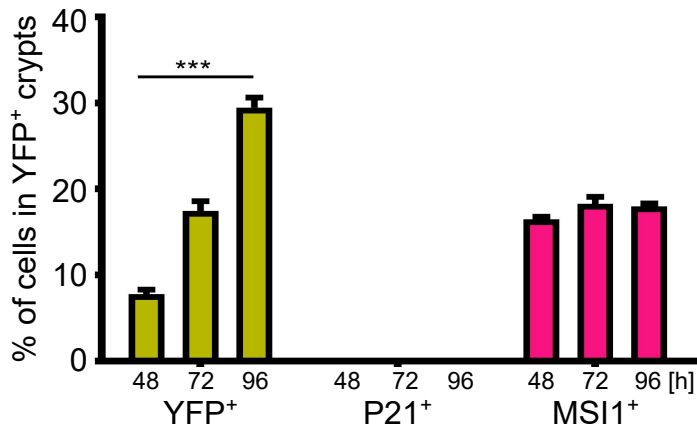
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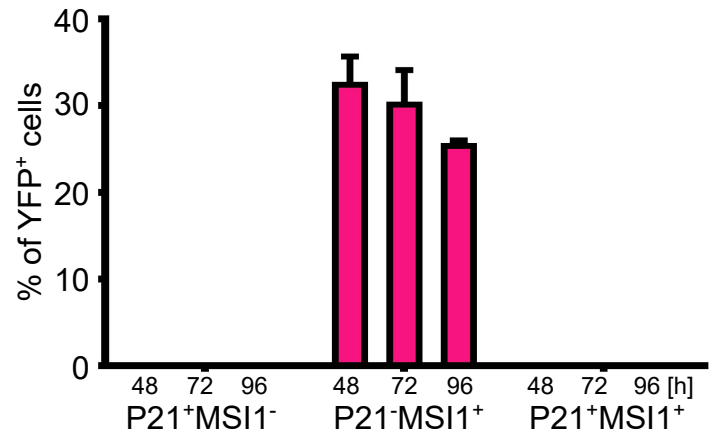
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B

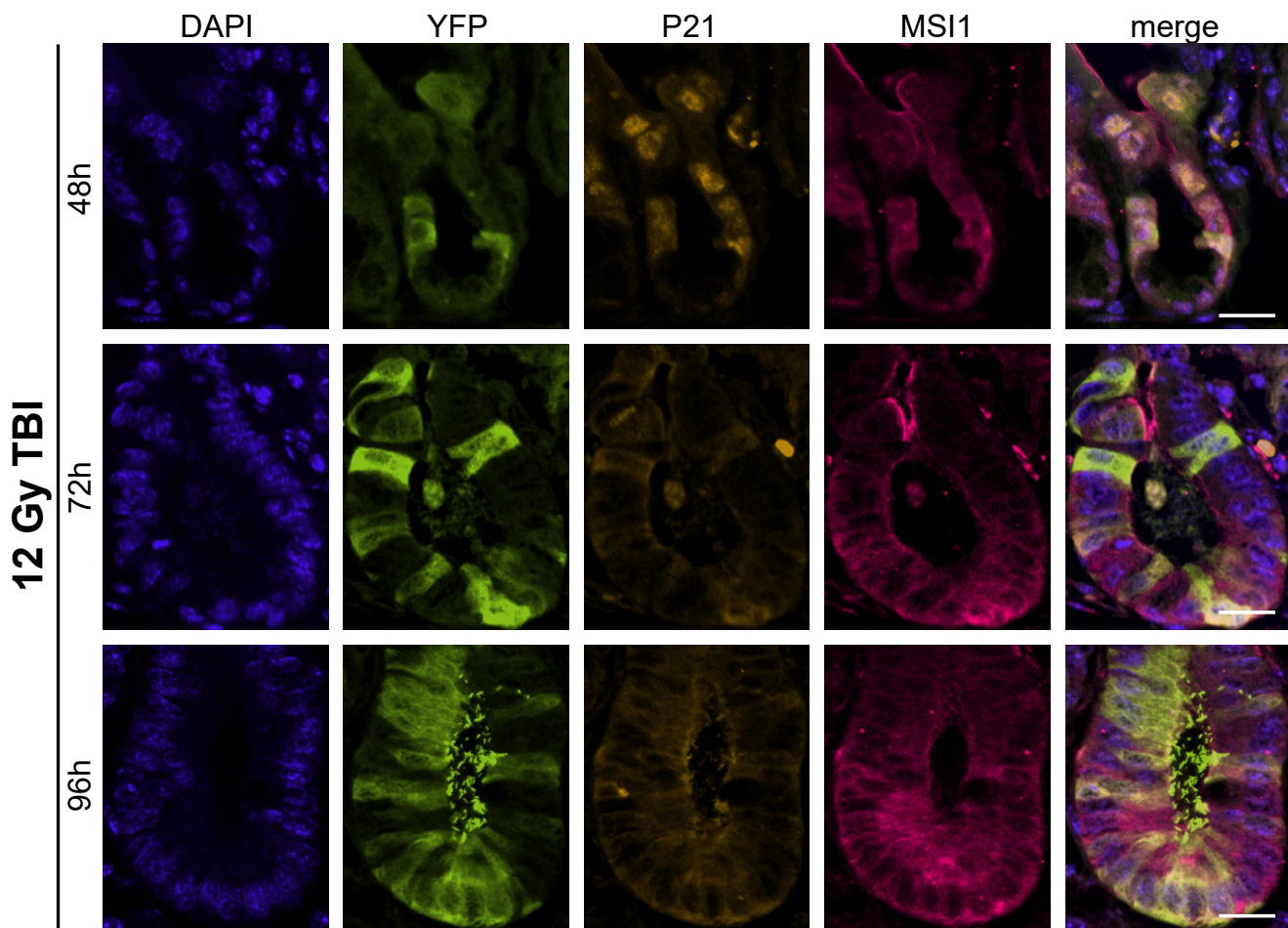


C

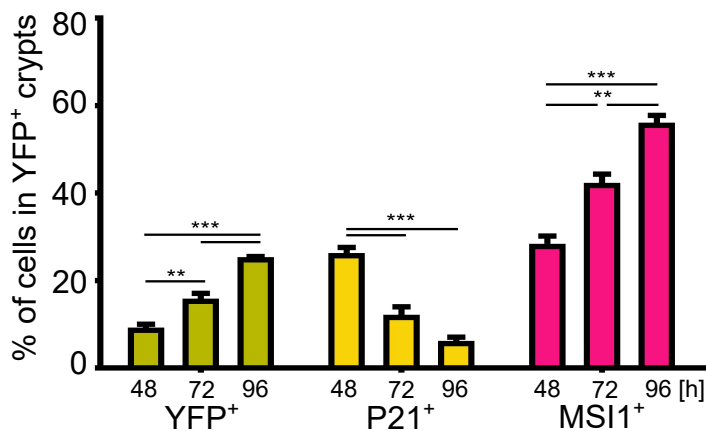


**Supplementary Figure 4.** Time-dependent p21<sup>Waf1/Cip1</sup> (P21) and MSI1 co-expression patterns in YFP<sup>+</sup> crypts after 0 Gy TBI of the *Bmi1<sup>Ctrl</sup>* mice treated according to protocol 2 (Supplementary Fig. 1B). (A) Representative IF images of DAPI, YFP, p21<sup>Waf1/Cip1</sup>, and MSI1 in the PSI crypts at 48, 72 and 96 h after tamoxifen injection obtained under a fluorescence microscope. The scale bar represents 20  $\mu$ m. (B) Quantification of the percentage of YFP<sup>+</sup>, p21<sup>Waf1/Cip1+</sup> or MSI1<sup>+</sup> cells in the YFP<sup>+</sup> crypts. (C) Quantification of the percentage of YFP<sup>+</sup> cells co-expressing p21<sup>Waf1/Cip1</sup>, MSI1 or p21<sup>Waf1/Cip1</sup> and MSI1 together. Data are represented as the mean  $\pm$  SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \*\*\* p < 0.001 by one-way ANOVA.

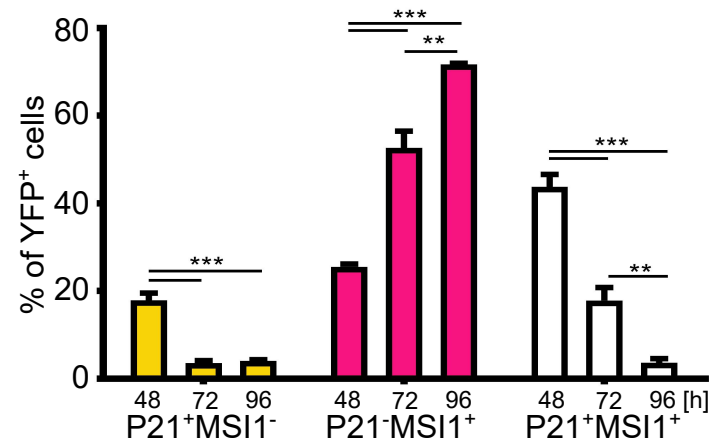
A

*Bmi1*<sup>Ctrl</sup>

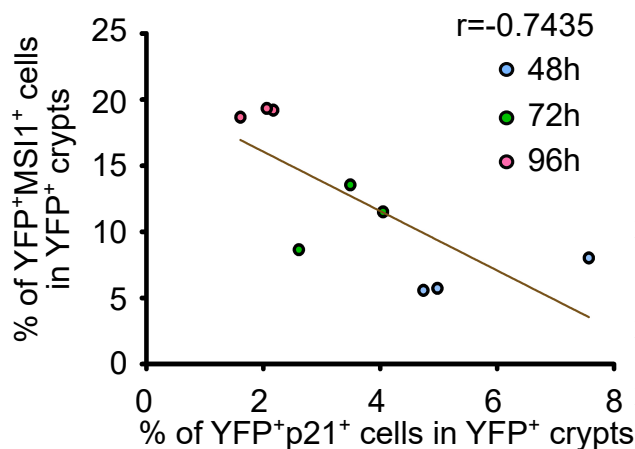
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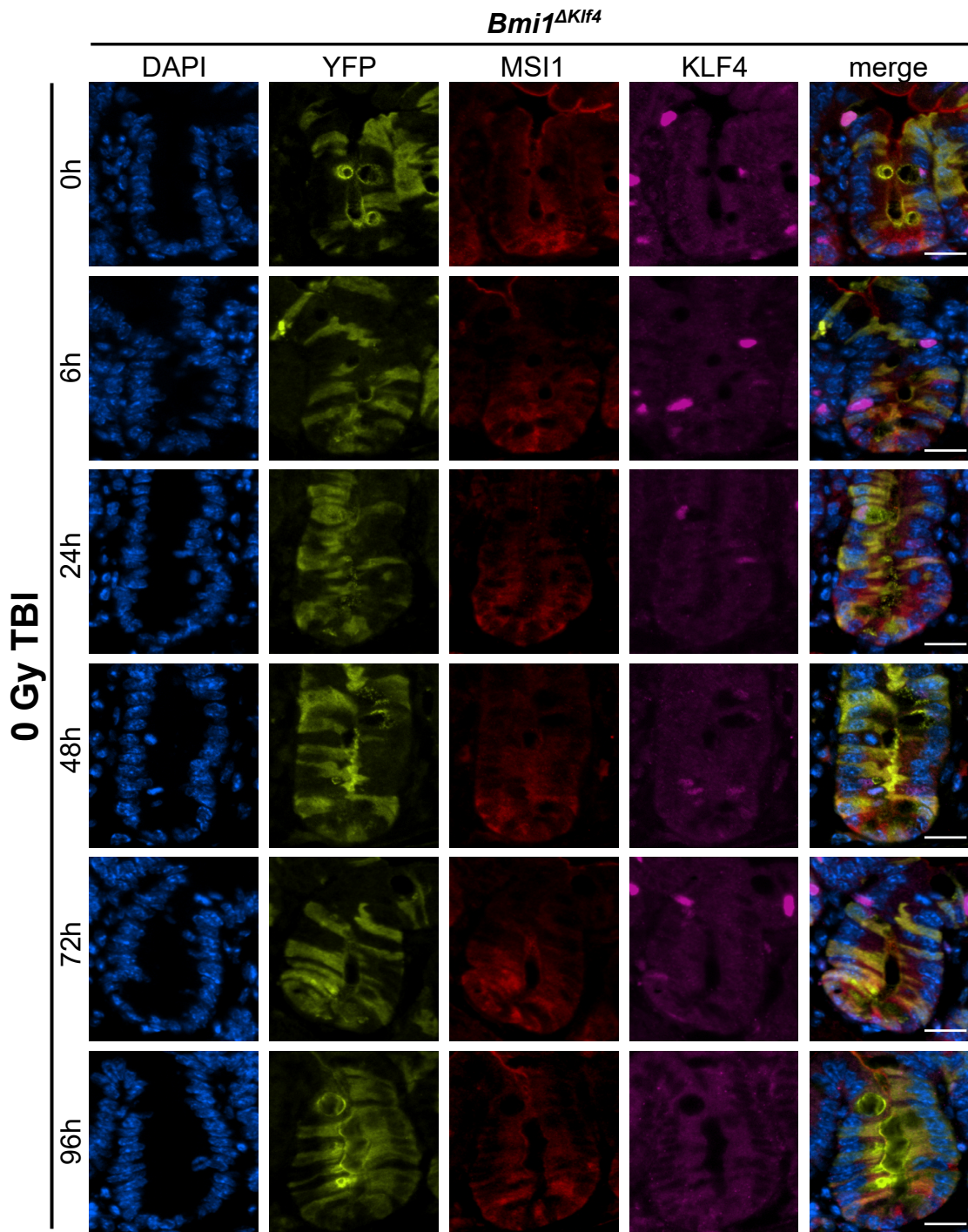
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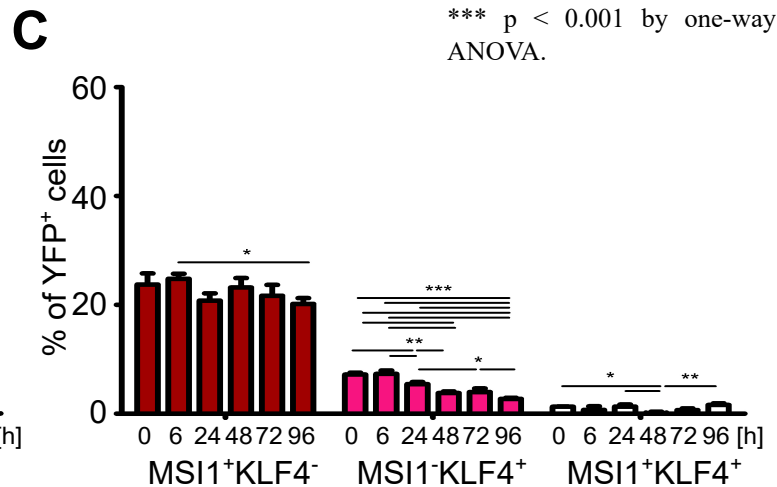
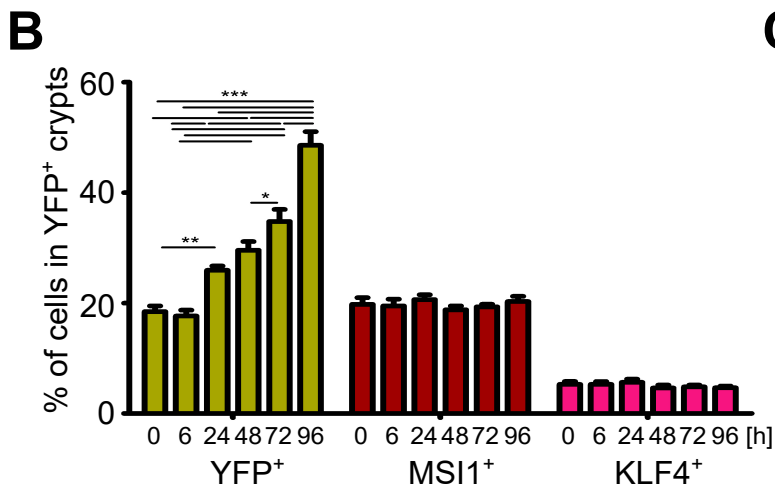
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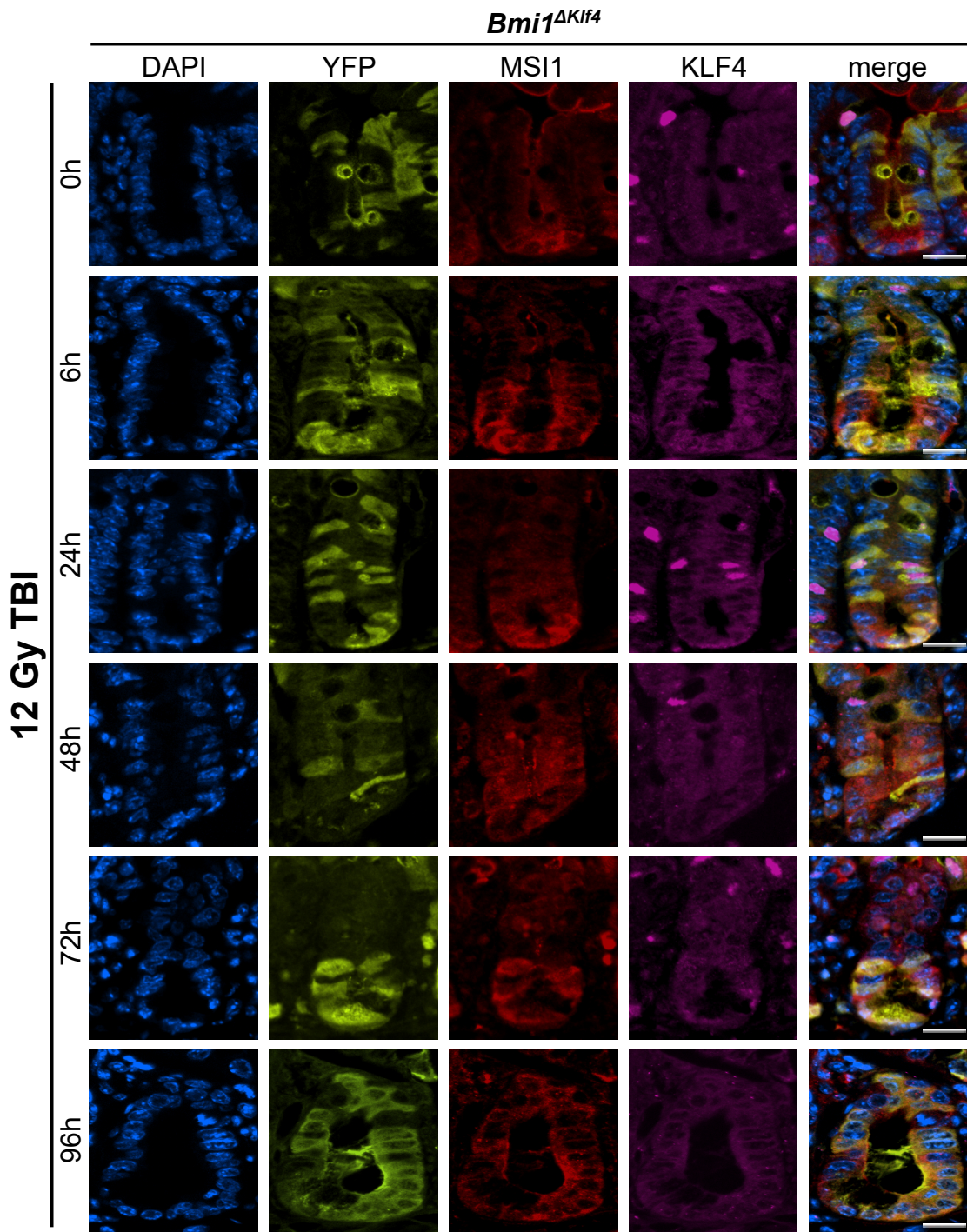


**Supplementary Figure 5.** Time-dependent p21<sup>Waf1/Cip1</sup> (P21) and MSI1 coexpression patterns in the YFP<sup>+</sup> crypts after 12 Gy TBI of the *Bmi1*<sup>Ctrl</sup> mice treated according to protocol 2 (Supplementary Fig. 1B). (A) Representative IF images of DAPI, YFP, p21<sup>Waf1/Cip1</sup>, and MSI1 in the PSI crypts at 48, 72 and 96 h after irradiation obtained under a fluorescence microscope. The scale bar represents 20  $\mu$ m. (B) Quantification of the percentage of YFP<sup>+</sup>, p21<sup>Waf1/Cip1</sup> or MSI1<sup>+</sup> cells in the YFP<sup>+</sup> crypts. (C) Quantification of the percentage of YFP<sup>+</sup> cells contained with p21<sup>Waf1/Cip1</sup>, MSI1 or p21<sup>Waf1/Cip1</sup> and MSI1 together. (D) Analysis of correlation between p21<sup>Waf1/Cip1</sup> and MSI1 expression in the YFP<sup>+</sup> crypts. Data are represented as the mean  $\pm$  SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and  $n = 3$  mice per group. \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  by one-way ANOVA. Analysis of correlation by Spearman correlation test.



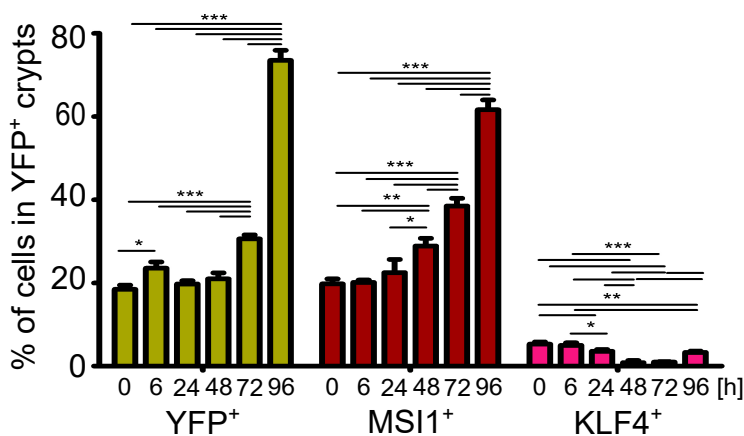
**Supplementary Figure 6.** Time-dependent MSI1 and KLF4 expression patterns in the YFP<sup>+</sup> crypts after 0 Gy TBI of the *Bmi1<sup>ΔKlf4</sup>* mice treated according to protocol 1 (Supplementary Fig. 1A). (A) Representative IF images of DAPI, YFP, MSI1, and KLF4 in the PSI crypts at 0, 6, 24, 48, 72 and 96 h after irradiation obtained under a fluorescence microscope. The scale bar represents 20 μm. (B) Quantification of the percentage of YFP<sup>+</sup>, MSI1<sup>+</sup> or KLF4<sup>+</sup> cells in the YFP<sup>+</sup> crypts. (C) Quantification of the percentage of YFP<sup>+</sup> cells costained with MSI1, KLF4 or MSI1 and KLF4 together. Data are represented as the mean ± SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 by one-way ANOVA.



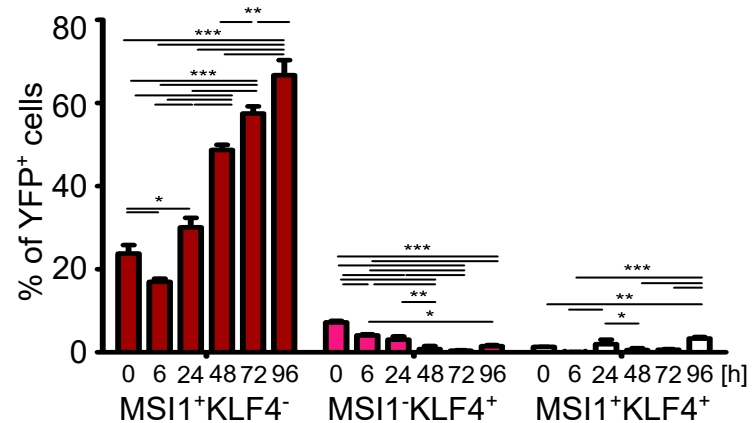


**Supplementary Figure 7.** Time-dependent MSI1 and KLF4 expression patterns in the YFP<sup>+</sup> crypts after 12 Gy TBI of the *Bmi1<sup>ΔKlf4</sup>* mice treated according to protocol 1 (Supplementary Fig. 1A). (A) Representative IF images of DAPI, YFP, MSI1, and KLF4 in the PSI crypts at 0, 6, 24, 48, 72 and 96 h after irradiation obtained under a fluorescence microscope. The scale bar represents 20 μm. (B) Quantification of the percentage of YFP<sup>+</sup>, MSI1<sup>+</sup> or KLF4<sup>+</sup> cells in the YFP<sup>+</sup> crypts. (C) Quantification of the percentage of YFP<sup>+</sup> cells costained with MSI1, KLF4 or MSI1 and KLF4 together. Data are represented as the mean ± SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 by one-way ANOVA.

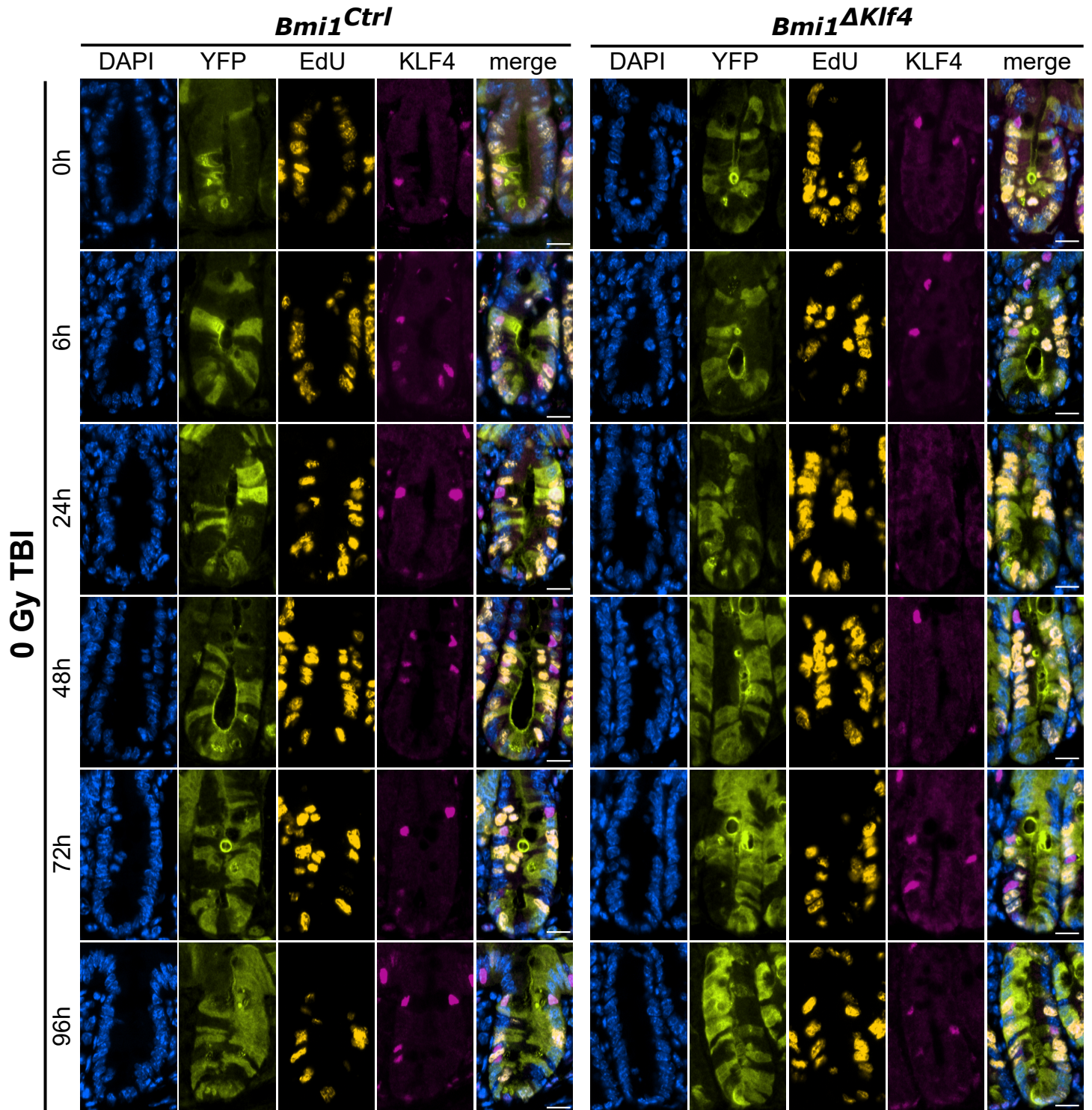
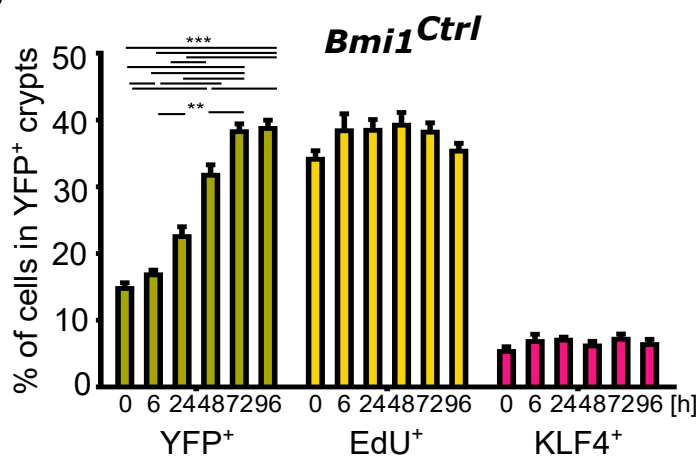
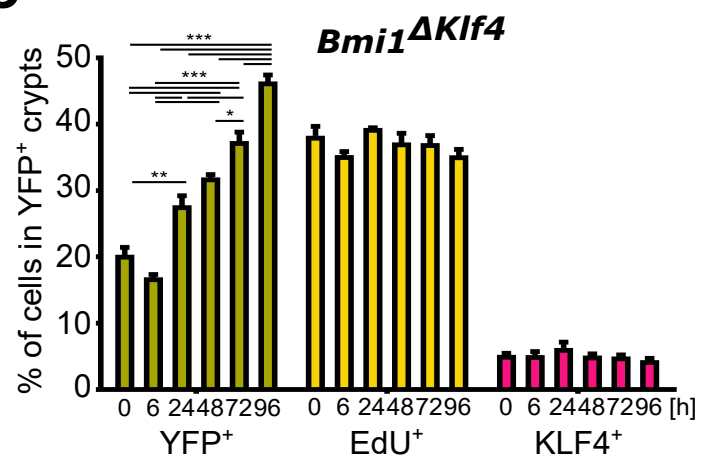
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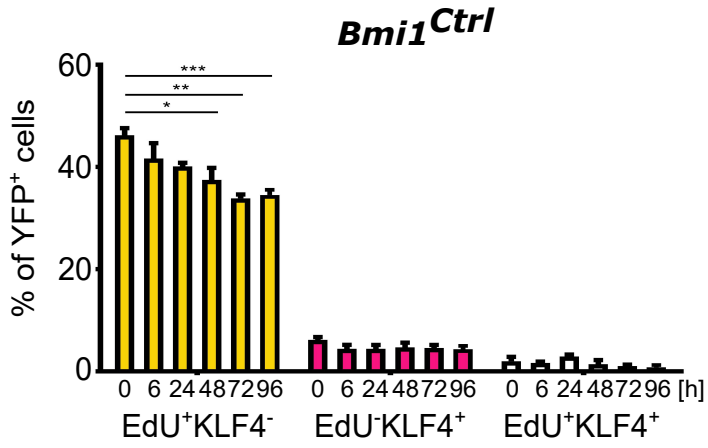
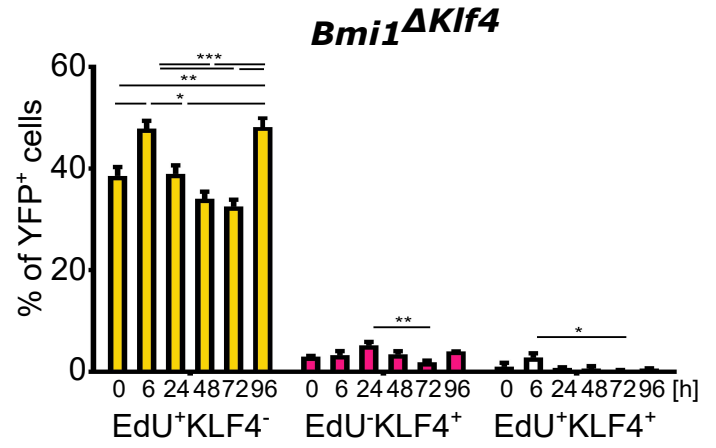
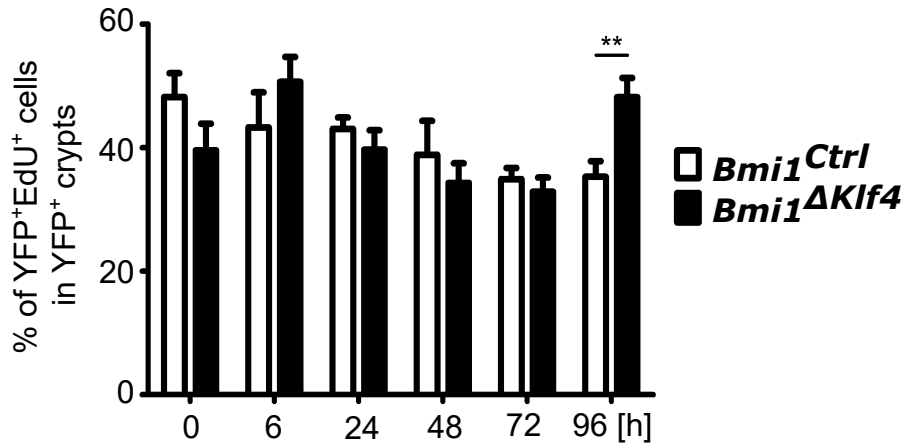


C

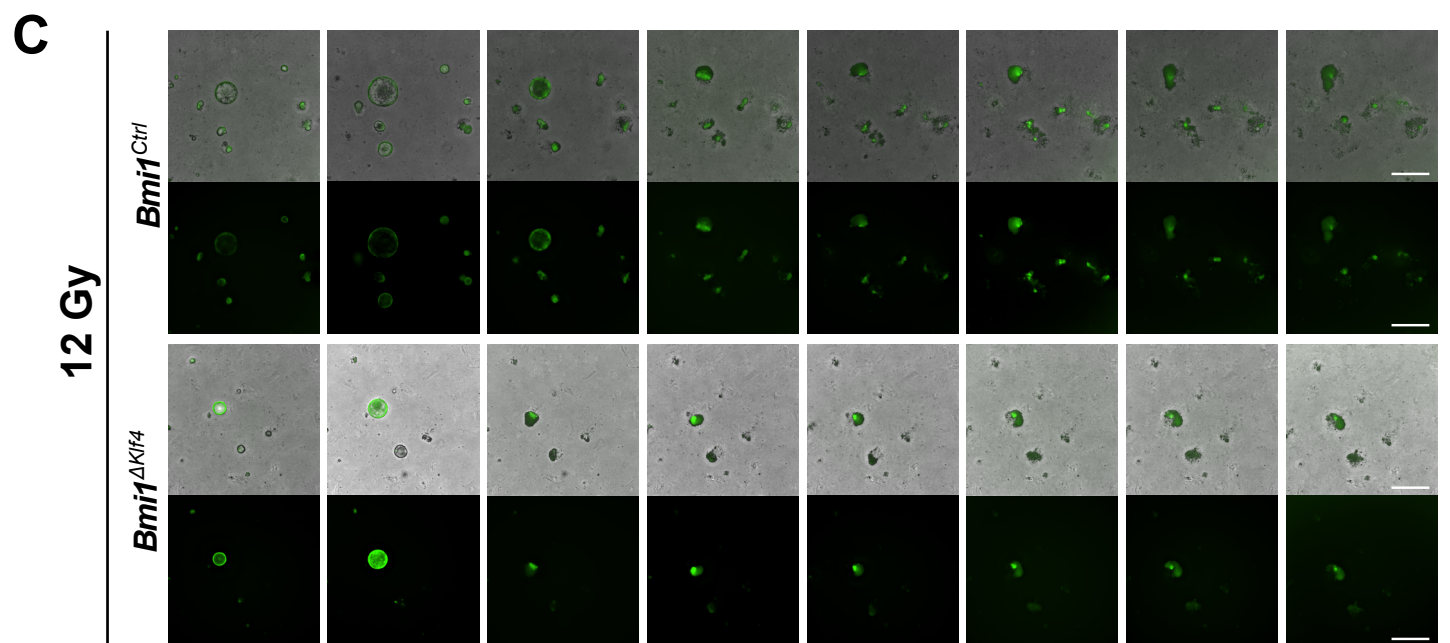
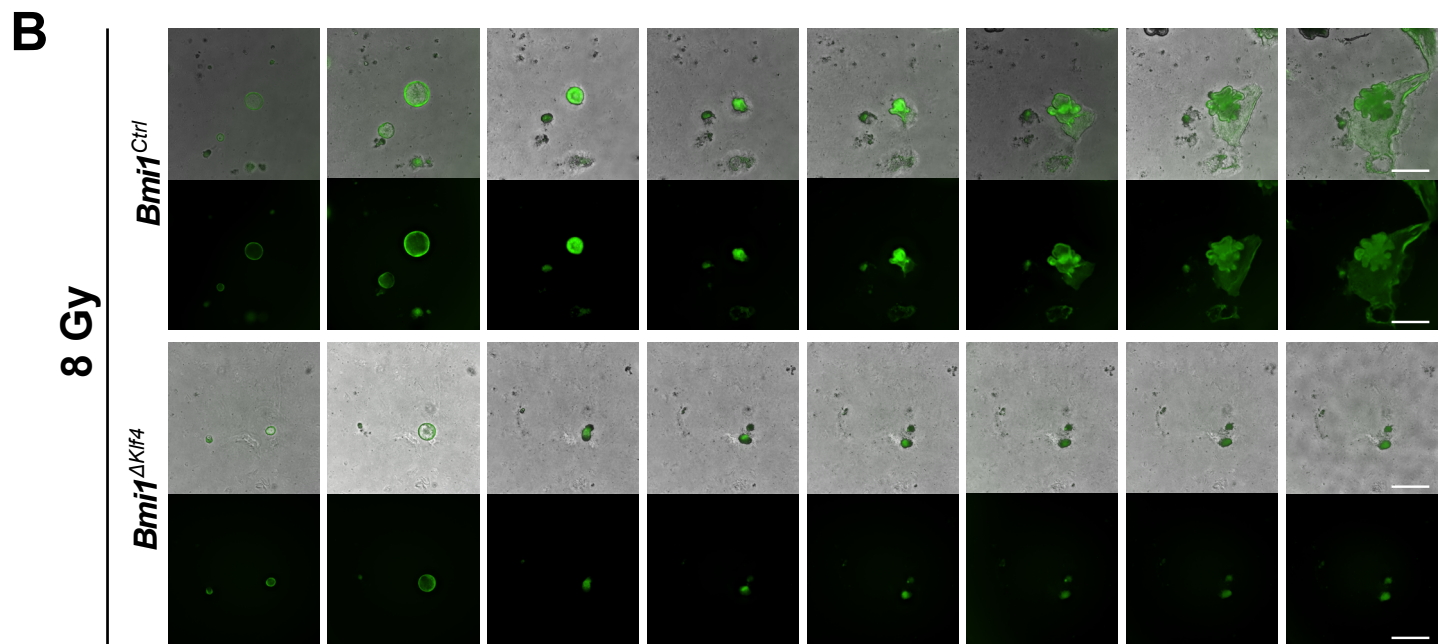
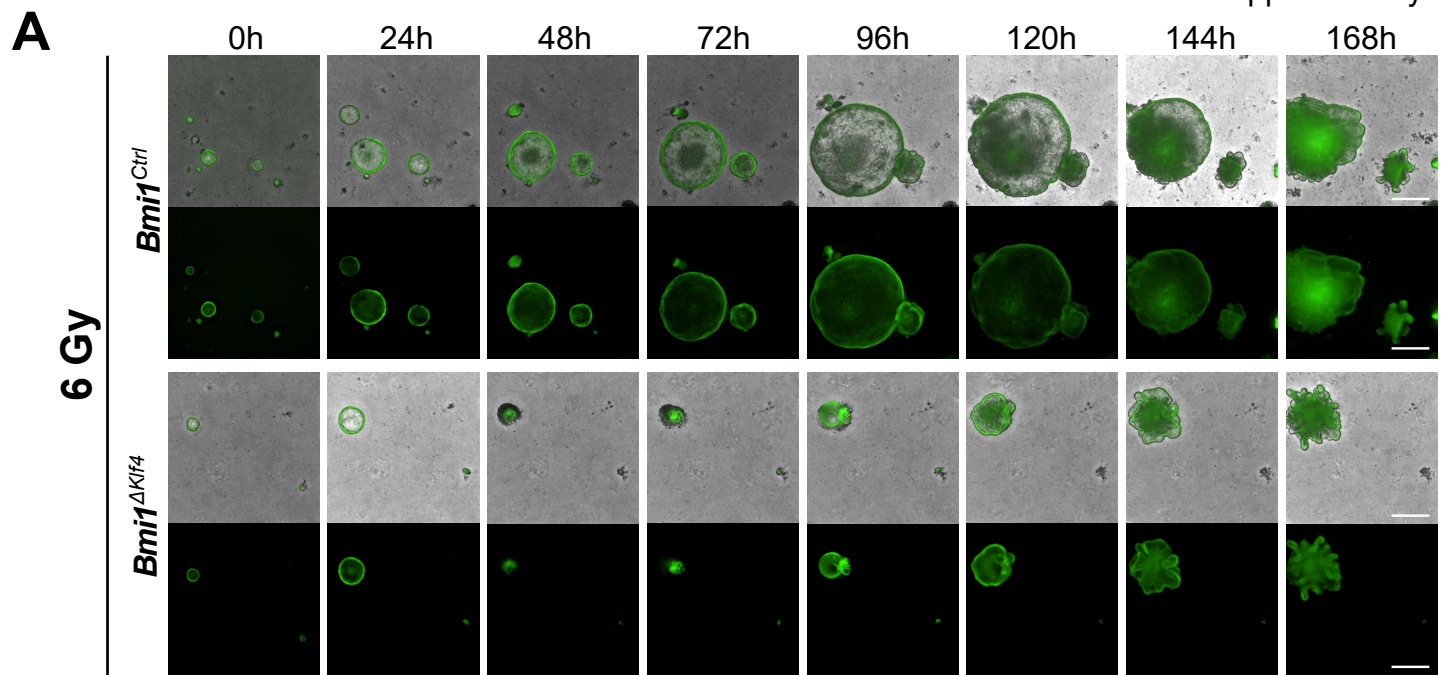




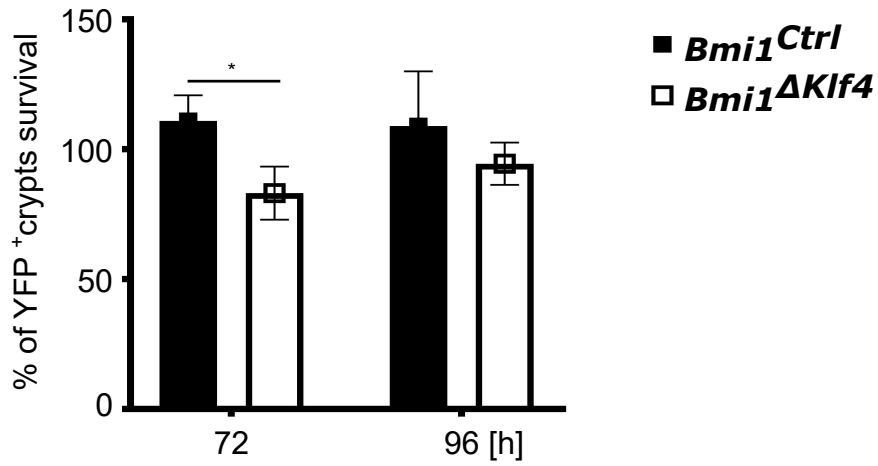
**A****B****C**

**D****E****F**

**Supplementary Figure 8.** KLF4 influences the proliferative ability of cells in the YFP<sup>+</sup> crypts after 0 Gy TBI of the *Bmi1<sup>Ctrl</sup>* and *Bmi1<sup>ΔKlf4</sup>* mice treated according to protocol 1 (Supplementary Fig. 1A). (A) Representative IF images of DAPI, YFP, EdU, and KLF4 in the PSI crypts at 0, 6, 24, 48, 72 and 96 h obtained under a fluorescence microscope. The scale bar represents 20 μm. (B-C) Quantification of the percentage of YFP<sup>+</sup>, EdU<sup>+</sup> or KLF4<sup>+</sup> cells in the YFP<sup>+</sup> crypts of the *Bmi1<sup>Ctrl</sup>* (B) and *Bmi1<sup>ΔKlf4</sup>* (C) mice. (D-E) Quantification of the percentage of YFP<sup>+</sup> cells costained with EdU, KLF4 or EdU and KLF4 together of the *Bmi1<sup>Ctrl</sup>* (D) and *Bmi1<sup>ΔKlf4</sup>* (E) mice. (F) Comparison of the percentage of YFP<sup>+</sup>EdU<sup>+</sup> cells in the YFP<sup>+</sup> crypts of the *Bmi1<sup>Ctrl</sup>* and *Bmi1<sup>ΔKlf4</sup>* mice. Data are represented as the mean ± SD, 20 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 by one-way ANOVA.



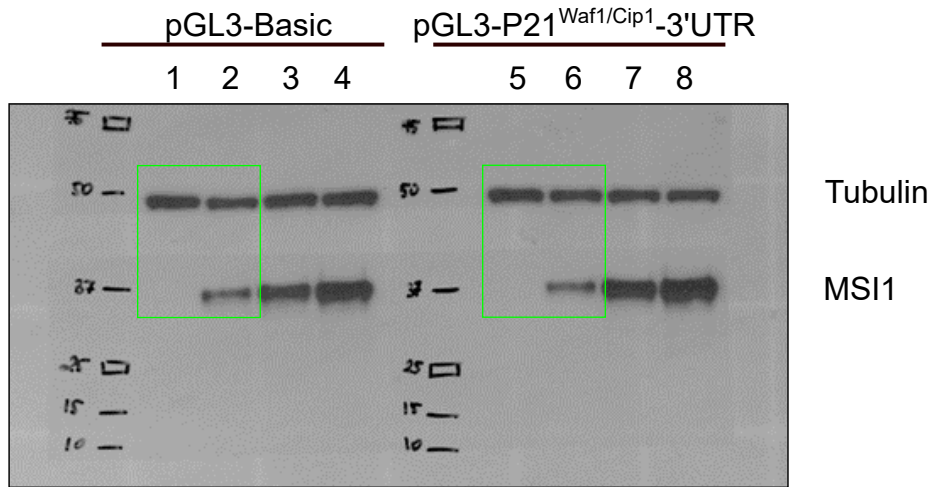
**Supplementary Figure 9.** KLF4 influences the YFP<sup>+</sup>-derived organoid formation and regenerative capability in response to  $\gamma$  radiation-induced injury. (A-C) Representative images of organoids derived from the FACS-sorted YFP<sup>+</sup> cells isolated from the *Bmi1<sup>Ctrl</sup>* and *Bmi1 <sup>$\Delta$ Klf4</sup>* mice at 0, 6, 24, 48, 72, 96, 120, 144 and 168 h after irradiation exposed to 6 Gy (A), 8 Gy (B) or 12 Gy (C) source of  $\gamma$  irradiation obtained under a fluorescence microscope. The lower panel represents fluorescent images, the upper panel represents merged images of bright-field and fluorescent images. The scale bar represents 500  $\mu$ m.

**A**

**Supplementary Figure 10.** Quantification of the YFP<sup>+</sup> crypts survival 72 h and 96 h post-irradiation of the *Bmi1*<sup>Ctrl</sup> and *Bmi1*<sup>ΔKlf4</sup> mice treated according to protocol 1 (Supplementary Fig. 1A) presented as the percentage of the YFP<sup>+</sup> proliferating crypts after irradiation vs. sham. Data are represented as the mean ± SD, 200 YFP<sup>+</sup> crypts were quantified per mouse, and n = 3 mice per group. \* p < 0.05 by Student's t-test.

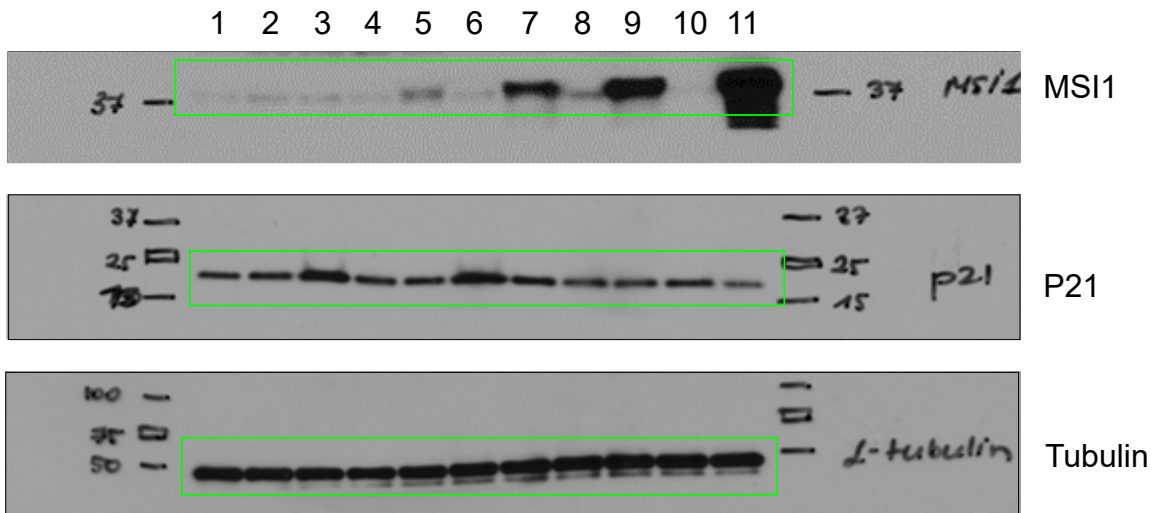
Uncropped blots in Fig. 3

Fig. 3C



HEK293T cells were transfected with 100 ng of pGL3-Basic (lanes 1-4) or pGL3-P21<sup>Waf1/Cip1</sup>-3'UTR (lanes 5-8). Additionally cells were transfected with 100 ng pReceiver-Lv216 (EV; lanes 1 and 5) or pCDH-CMV-Msi1 100 ng (lanes 2 and 6), 150 ng (lanes 3 and 7), and 200 ng (lanes 4 and 8). Protein ladder was marked.

Fig. 3H

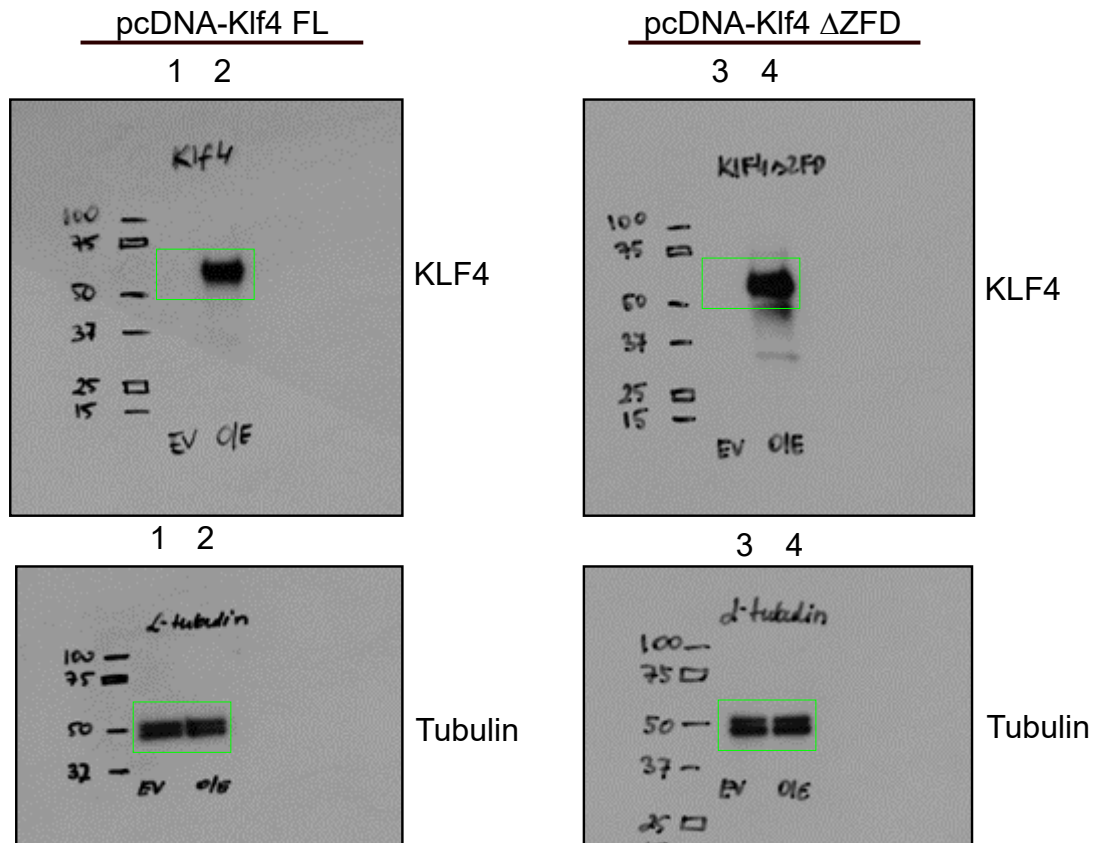


Lane	1	2	3	4	5	6	7	8	9	10	11
12 Gy	-	-	+	-	-	+	+	-	-	+	+
<i>MSI1</i> O/E	-	-	-	-	+	-	+	-	+	-	+

HEK293T cells were irradiated with dose of 12 Gy or remained non-treated and 24 h later MSI1 was expressed using 100 ng of pCMV6-AC-GFP-MSI1 encoding human MSI1. Samples without MSI1 overexpression were transfected with 100 ng of pcDNA3.1 used as an empty vector (EV) control.

Uncropped blots in Fig. 5

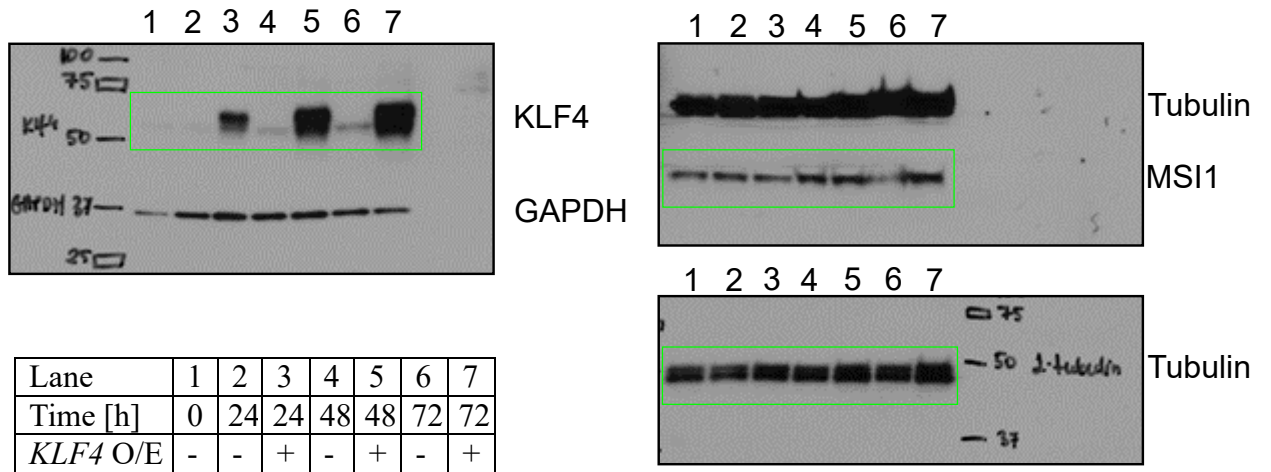
Fig. 5B



HEK293T cells were transfected with 100 ng of pcDNA3.1 (EV, empty vector control, lanes 1 and 3) or 100 ng of pcDNA3.1-Klf4 FL coding mouse full length *Klf4* (lane 2) or pcDNA3.1-Klf4 DZFD coding mouse *Klf4* mutant with deletion of C-terminal DNA-binding domains and encoding aminoacids from 1 to 349 of the full length protein (lane 4).

Uncropped blots in Fig. 5

Fig. 5F

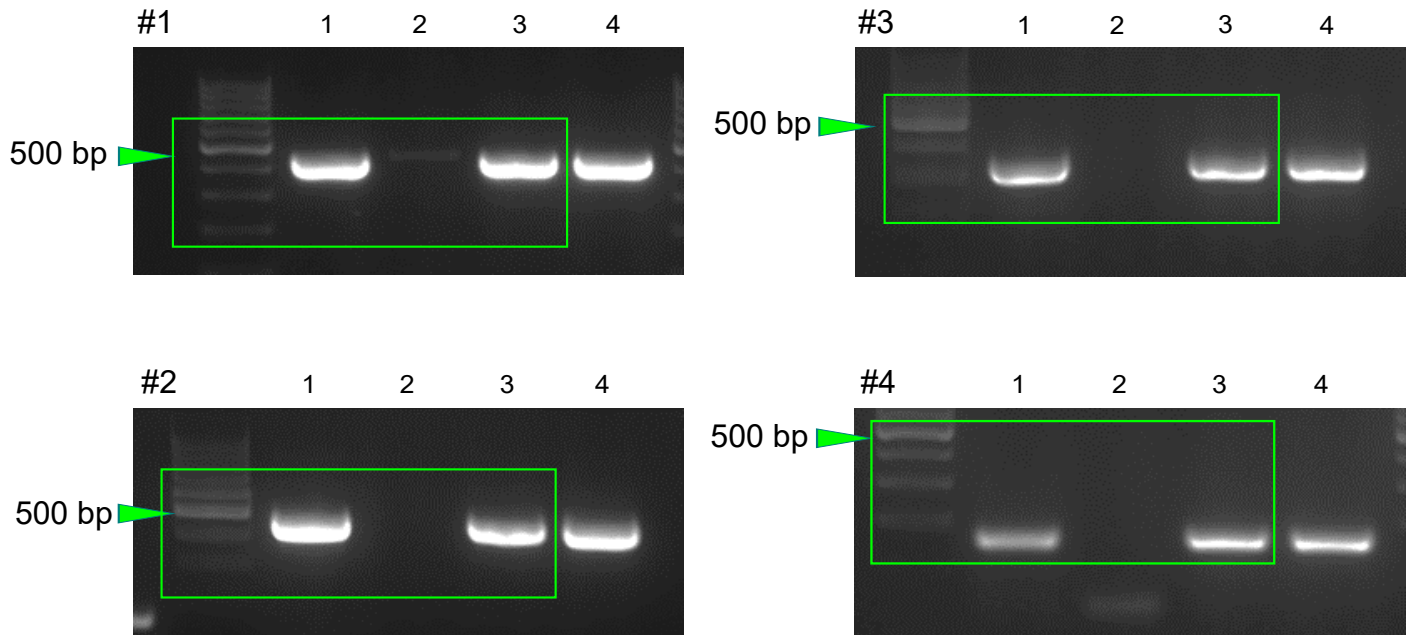


HEK293T cells were transfected with 100 ng of pcDNA3.1 (EV, empty vector control, lanes 1, 2, 4 and 6) or 100 ng of pcDNA3.1-KLF4 FL coding human full length *KLF4* (lanes 3, 5 and 7). For tubulin both shorter and longer exposures are shown.



Uncropped gels in Fig. 5

Fig. 5I



Lane:

1. input
2. IgG
3. KLF4 Ab 10  $\mu$ L
4. KLF4 Ab 15  $\mu$ L