



Ionizing radiation Increases Type I Collagen Matrix Production and may Contribute to Pulmonary Fibrosis

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Introduction

- Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease resulting from chronic lung scarring.
- IPF fibroblasts relentlessly produce type 1 collagen, which is the primary extracellular matrix (ECM) found in IPF patient lung tissues, promoting lung fibrosis.
- IPF fibroblasts are anti-apoptotic and proliferative in response to type 1 collagen.
- IPF fibroblasts show increased DNA damage repair in response to ionizing radiation, demonstrating their unique death-resistant properties in response to genotoxic insults.

Hypotheses

- Our hypothesis is that ionizing radiation triggers increased type 1 collagen production in highly viable IPF fibroblasts compared to non-IPF lung fibroblasts and bronchial epithelial cells (HBEC-3KT) due to enhanced DNA repair mechanisms.
- Abnormally viable IPF fibroblasts contribute to the development of lung fibrosis

Methods

- IPF fibroblasts, non-IPF fibroblasts and HBEC-3KT were seeded on pure collagen (PC) coated dishes and treated with radiation (0 and 9 Gy) 24 hours later.
- Cell viability assay was conducted 5 days after radiation treatment.
- Conditioning media was collected following radiation to measure extracellular type 1 collagen production in irradiated IPF and non-IPF fibroblasts.
- Western blot, Sircol assay and Real Time-PCR were performed to measure Col1A1 protein, newly formed collagen and Col1A1 mRNA levels, respectively, in irradiated (1 x 15Gy, 4 x 8Gy) and unirradiated murine lung tissue.

Results

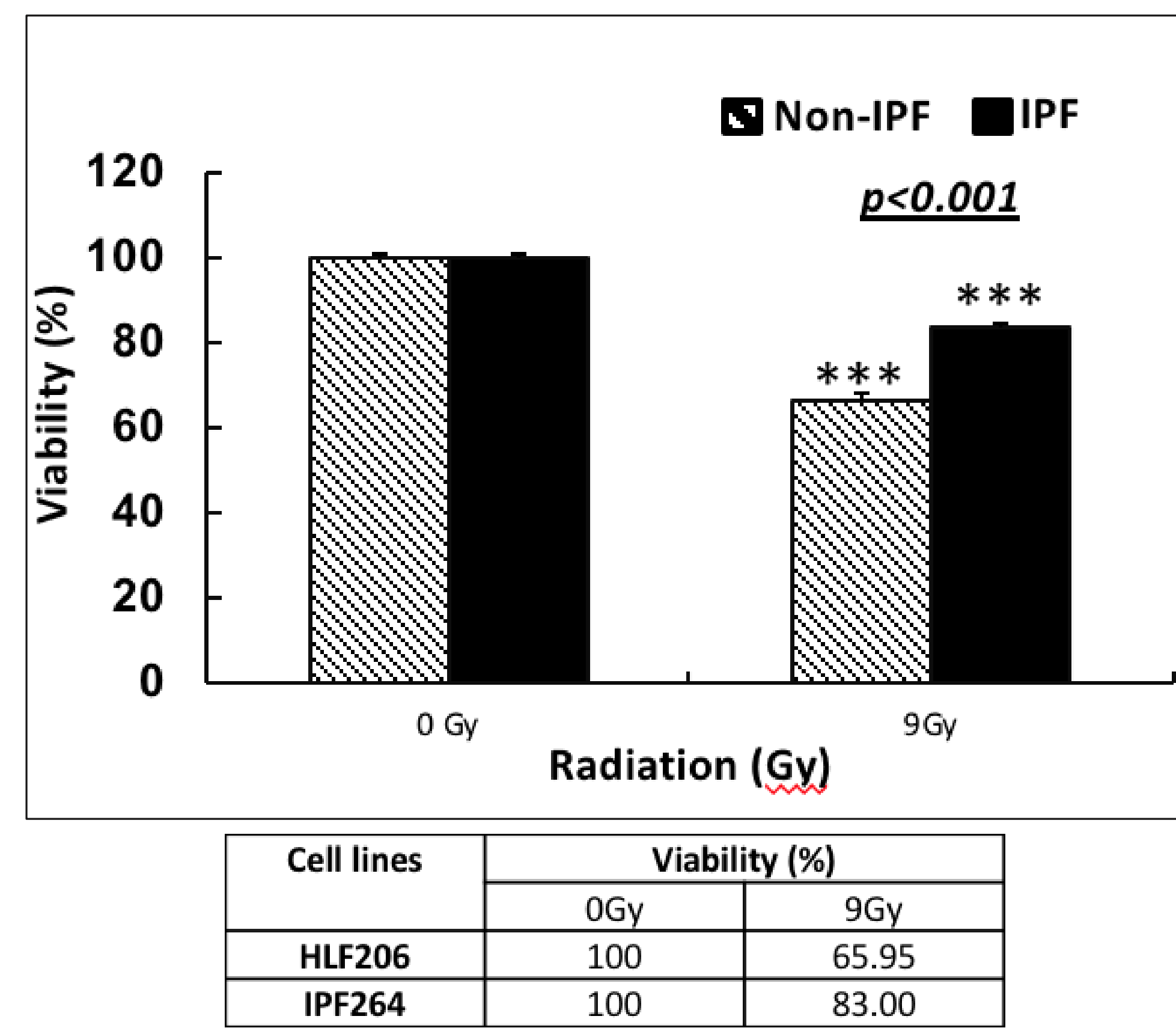


Figure 1. Comparison of IPF and non-IPF fibroblasts viability (%) 5 days after 0Gy and 9Gy radiation.

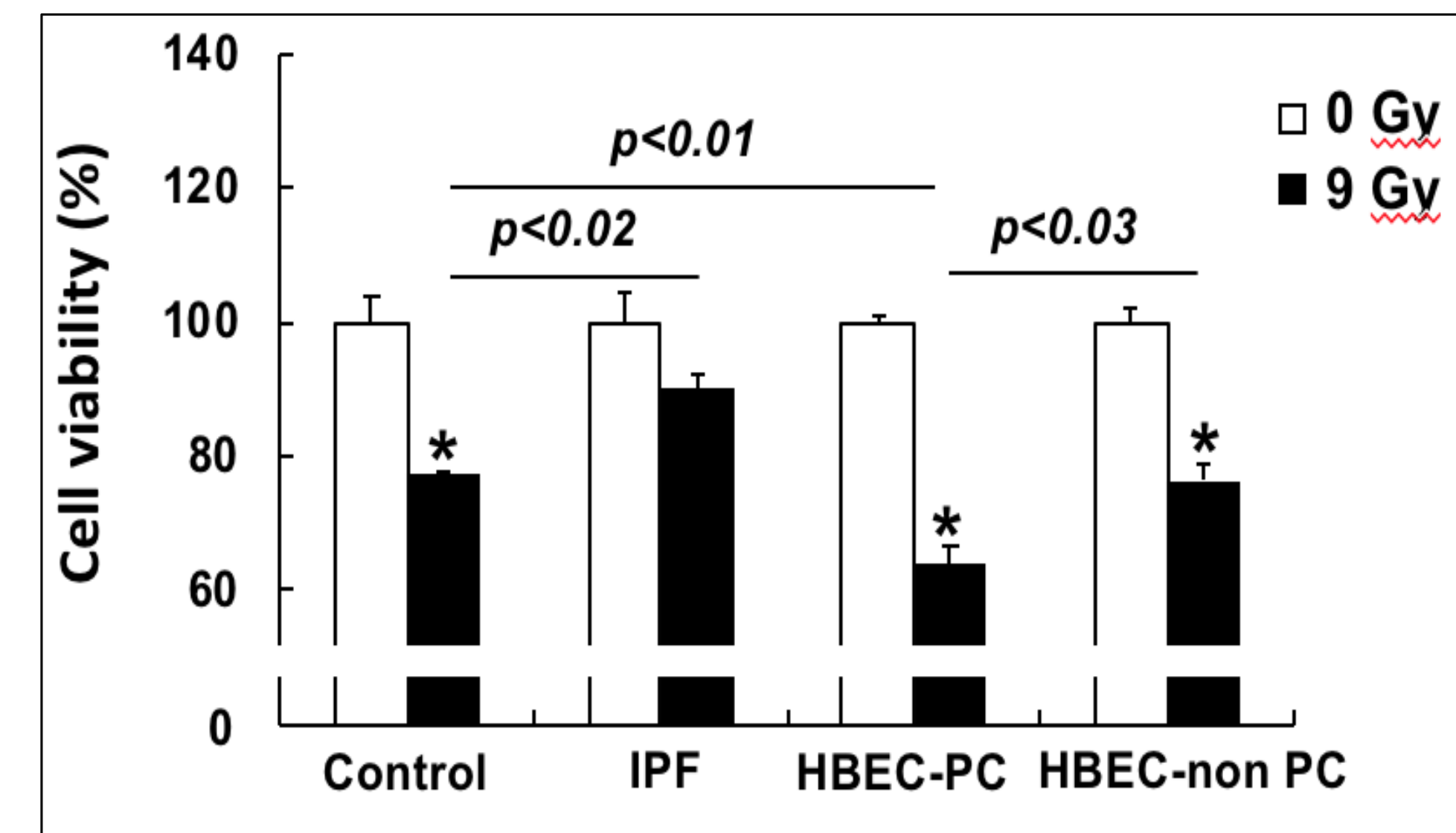


Figure 2. Viability (%) of human bronchial epithelial cells (HBEC-3KT), IPF and non-IPF fibroblasts 3 days after 9Gy radiation.

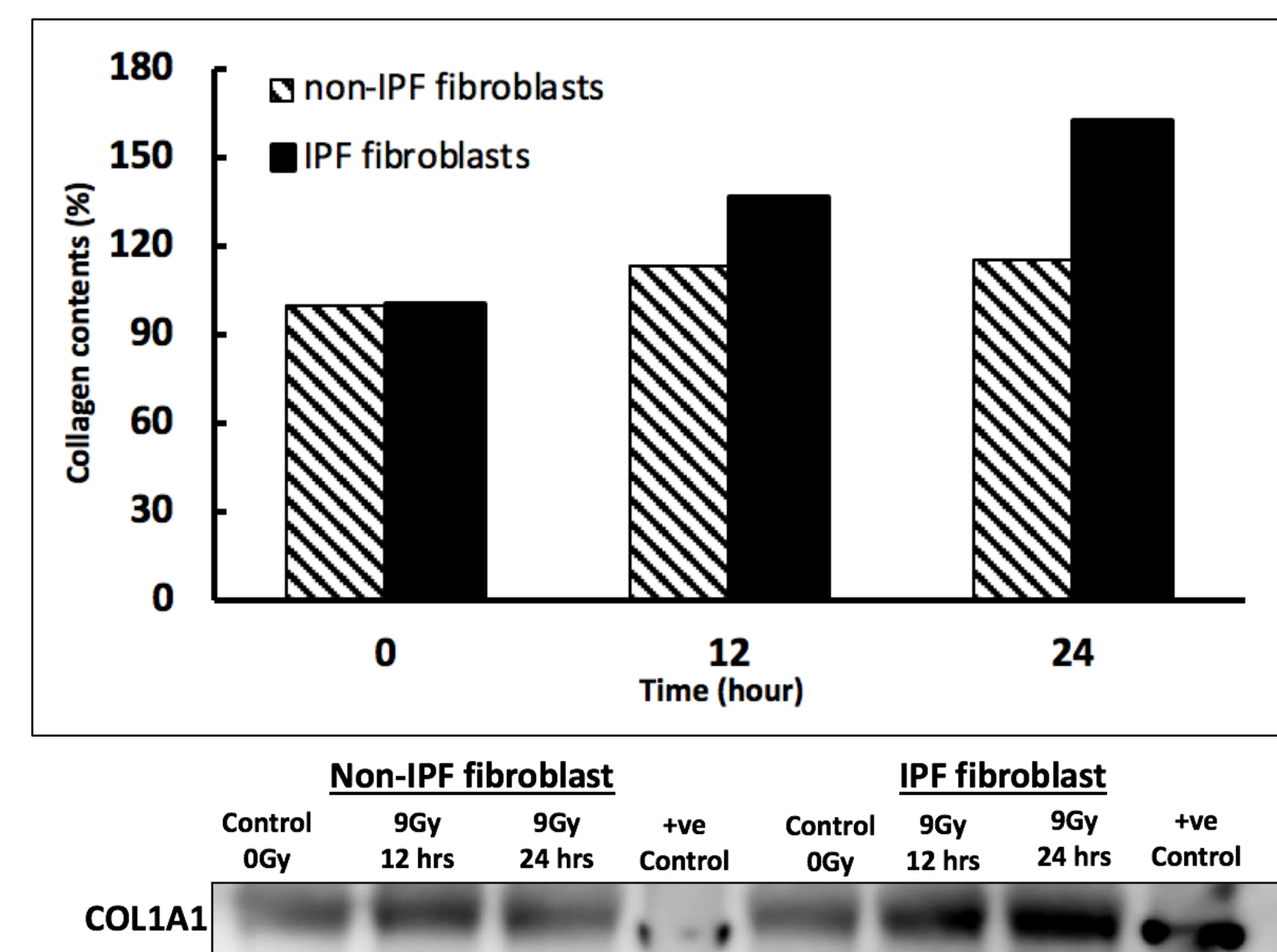


Figure 3. Western blot analysis of type 1 collagen production in conditioning media in IPF and non-IPF fibroblasts 0, 12 and 24 hours after 9Gy radiation.

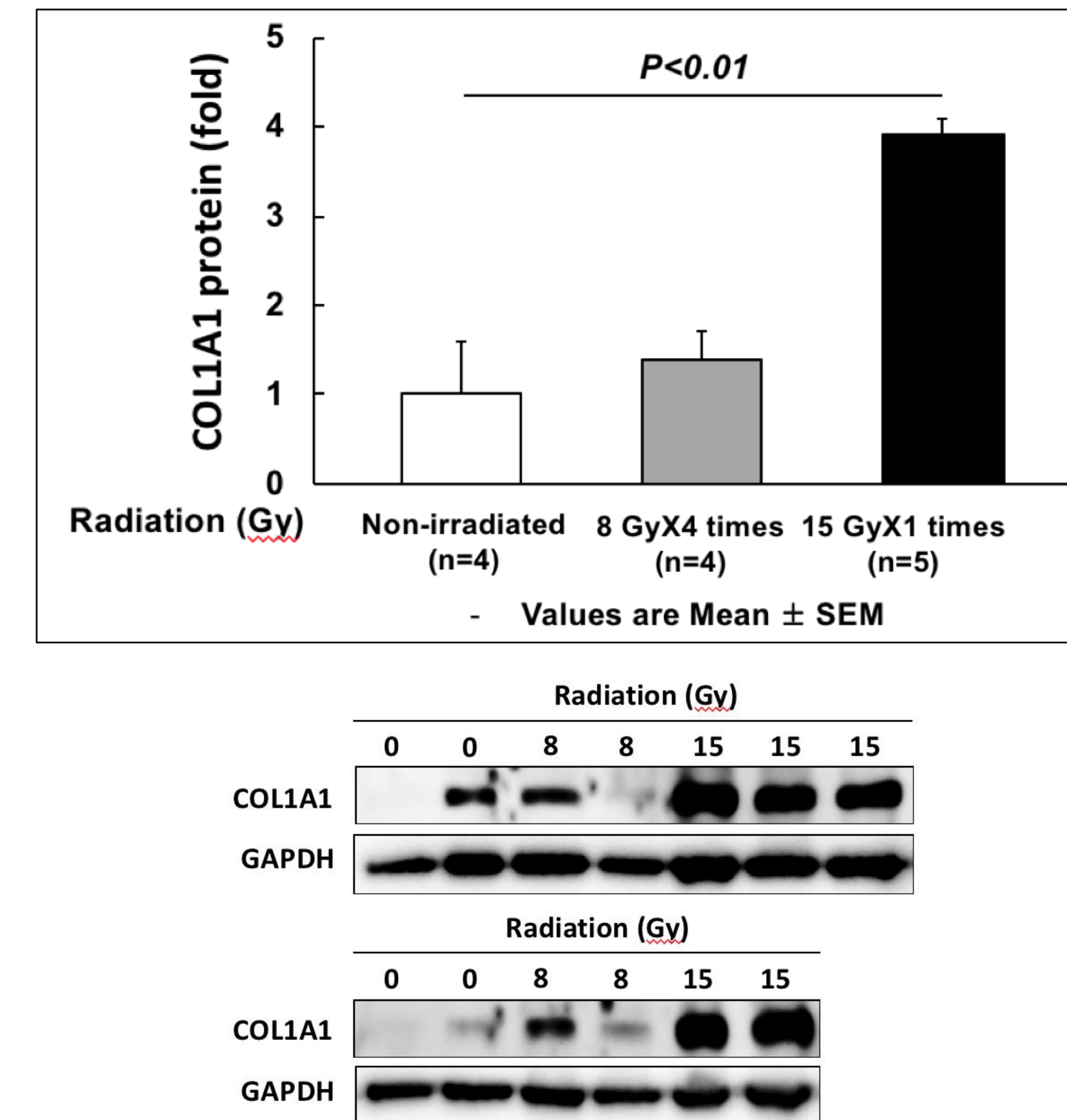


Figure 4. Western Blot analysis of type 1 collagen production in irradiated murine lung tissues after radiation. Unirradiated lung tissue was used as control.

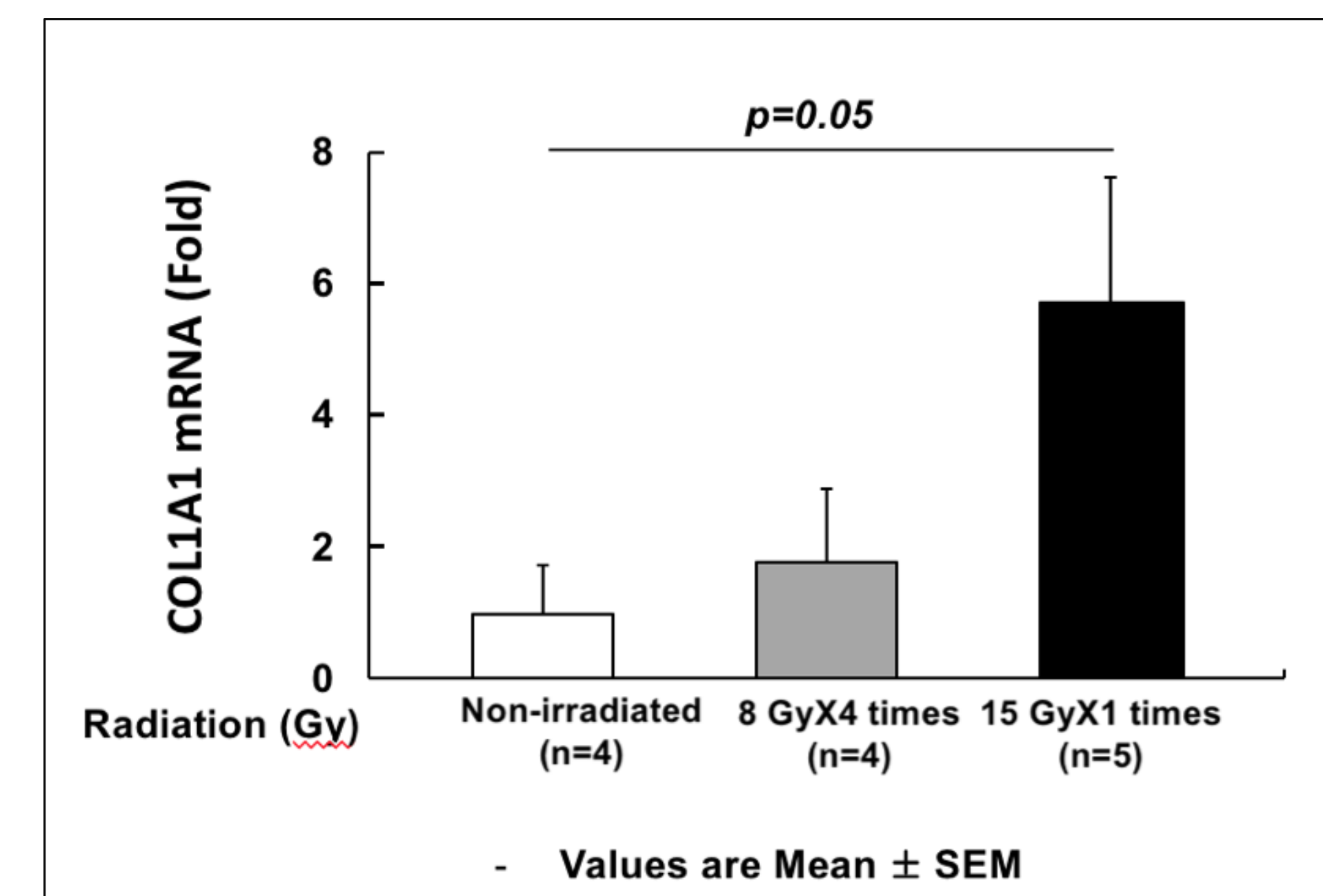


Figure 5. Real Time-PCR analysis of in vivo mRNA expression in irradiated murine lung tissues.

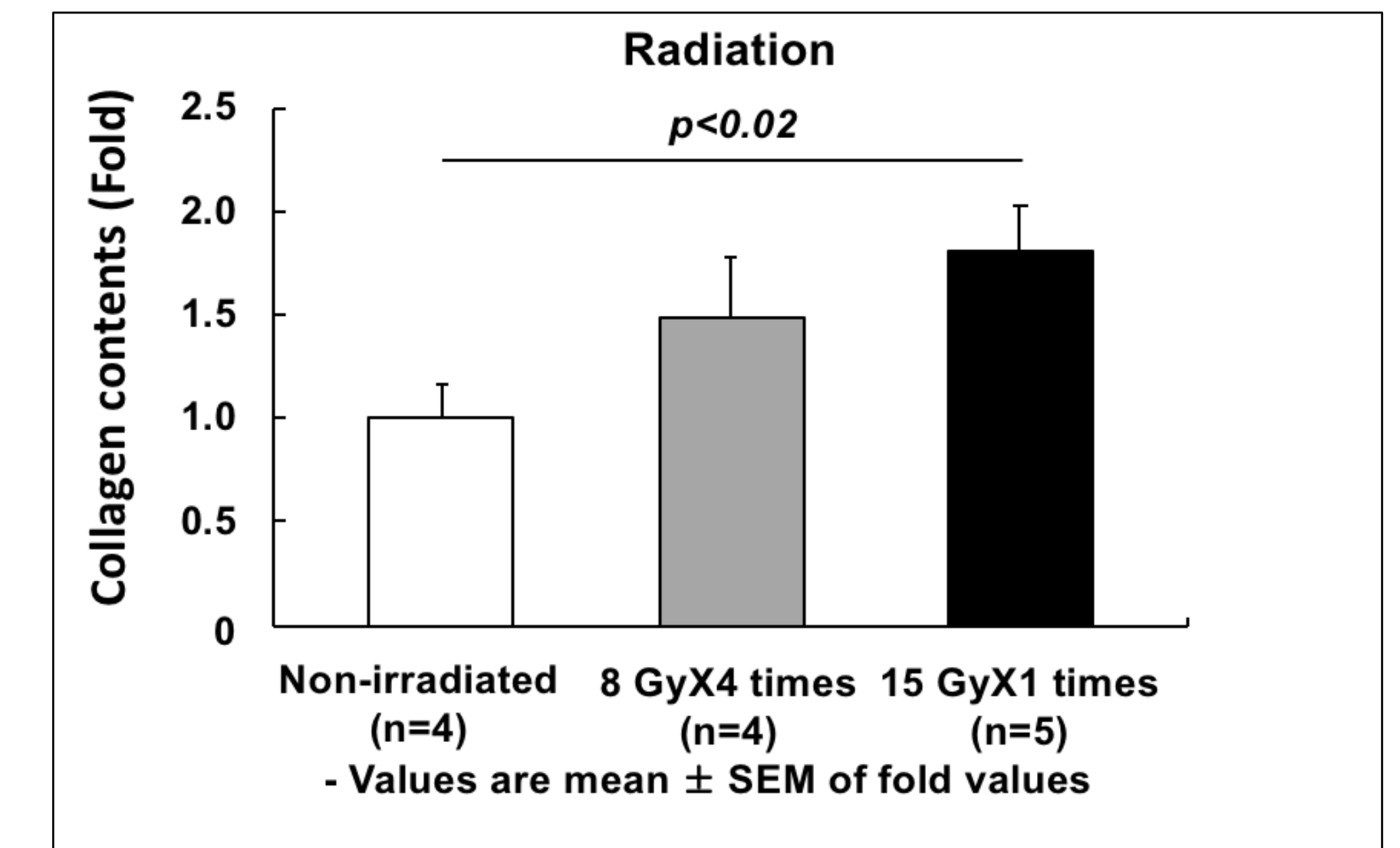


Figure 6. Sircol assay results in irradiated murine lung tissues compared to unirradiated controls.

Conclusions

- Irradiated IPF fibroblasts are more viable compared to irradiated non-IPF fibroblasts and HBEC-3KT cells on collagen matrix.
- Preliminary Western blots indicate irradiated IPF fibroblasts may increase type 1 collagen production compared to non-IPF fibroblasts.
- Irradiated murine lung tissue has increased type 1 collagen ECM and mRNA production.
- Our results suggest that the presence of radio-resistant fibrotic fibroblasts may promote lung fibrosis.

Future Goals

- Determine the pathological role of microRNA-29 in regulating type 1 collagen extracellular matrix production in IPF fibroblasts .
- Evaluate global underlying mechanisms that contribute to both IPF and radiation-induced pulmonary fibrosis (RIPF) development.

Proposed Model

