BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-21-191

Title: SOCS1 counter-acts the ROS-mediated survival signals and promotes apoptosis with cell cycle modulation to increase radiosensitivity of colorectal cancer cells

Article Type: Article

Keywords: SOCS1; Radiation Stress; Cell Cycle Modulation; Reactive Oxygen Species; Cell Survival Signaling

Corresponding Author: Choong-Eun Lee

Authors: Ji-Yoon Ryu[1], Jiyoung Oh[1], Su-Min Kim[1], Won-Gi Kim[1,2], Hana Jeong[1], Seol-Hee Kim[1], Sinae Ahn[1], Byong Chul Yoo[2], Choong-Eun Lee[1,*]

Institution: [1]Department of Biological Science, Sungkyunkwan University, Korea,
[2]Colorectal Cancer Branch, Research Center, National CAncer Institute, Korea,

SOCS1 counteracts ROS-mediated survival signals and promotes apoptosis by modulating cell cycle to increase radiosensitivity of colorectal cancer cells

Ji-Yoon Ryu[1], Jiyoung Oh[1], Su-Min Kim[1], Won-Gi Kim[1, 2], Hana Jeong[1], Shin-Ae Ahn[1], Seol-Hee Kim[1], Ji-Young Jang [3], Byong Chul Yoo [2], Chul Woo Kim[3], and Choong-Eun Lee[1]*

[1] Laboratory of Immunology, Department of Biological Science, College of Natural Science, Sungkyunkwan University, Suwon 16419 Korea, [2]Colorectal Cancer Branch, Research Center, National Cancer Institute, Goyang, 10408 Korea, [3]Tumor Immunity Medical Research Center, Cancer Research Institute, Seoul National University College of Medicine, Seoul, 03080 Korea

Running Title: Radiosensitization and cell cycle modulation by SOCS1

This study was supported in part by NRF grants #20100018682, #2013M2B2A9A03051275, #2015003291, #2016911262, #2018R1A2B6002201, and 2022R1H1A2003471. H Jeong was also supported in part by 2017 Global Ph.D Fellowship

* Corresponding authors

Choong-Eun Lee, Department of Biological Science, College of Natural Science, Sungkyunkwan University, Suwon, 440-746 Korea. e-mail: celee@skku.edu. Tel:82-31-270-7006

Keywords: SOCS1, Radiation Stress, Cell Cycle Modulation, Reactive Oxygen Species, Cell Survival Signaling

Abstract

As negative regulators of cytokine signaling pathways, suppressors of cytokine signaling (SOCS) proteins have been reported to possess both pro-tumor and anti-tumor functions. Our recent studies have demonstrated suppressive effects of SOCS1 on epithelial to mesenchymal signaling in colorectal cancer cells in response to fractionated ionizing radiation or oxidative stress. The objective of the present study was to determine the radiosensitizing action of SOCS1 as an anti-tumor mechanism in colorectal cancer cell model. In HCT116 cells exposed to ionizing radiation, SOCS1 over-expression shifted cell cycle arrest from G2/M to G1 and promoted radiation-induced apoptosis in a p53-dependent manner with down-regulation of cyclin B and up-regulation of p21. On the other hand, SOCS1 knock-down resulted in a reduced apoptosis with a decrease in G1 arrest. The regulatory action of SOCS1 on the radiation response was mediated by inhibition of radiation-induced Jak3/STAT3 and Erk activities, thereby blocking G1 to S transition. Radiation-induced early ROS signal was responsible for the activation of Jak3/ Erk/STAT3 that led to cell survival response. Our data collectively indicate that SOCS1 can promote radiosensitivity of colorectal cancer cells by counteracting ROS-mediated survival signal, thereby blocking cell cycle progression from G1 to S. The resulting increase in G1 arrest with p53 activation then contributes to the promotion of apoptotic response upon radiation. Thus, induction of SOCS1 expression may increase therapeutic efficacy of radiation in tumors with low SOCS1 levels.

I. Introduction

Initially identified as negative regulators of cytokine signal transduction, suppressors of cytokine signaling (SOCS) have been widely studied for their anti-inflammatory and growth-inhibitory actions in immune scenarios and malignant conditions (1). Earlier studies have shown that SOCS genes are silenced in various tumors by promoter DNA methylation, the reversal of which will result in the suppression of tumor cell growth (2-4). These findings have triggered investigations on the anti-tumor function of SOCS in diverse systems and revealed differential actions of SOCS in isoform- and cell type-specific manners (5-8). In several prostate cancer cell lines, SOCS1 and SOCS3 can act as negative growth regulators of IL-6 and androgen, respectively (6, 7). SOCS1 and SOCS3 both can promote Fas-induced apoptosis of leukemic T cells through down-regulation of cell survival pathways by NF-κB and Bfl-1 (5). More recently, we and others have demonstrated the negative regulation of epithelial to mesenchymal transition (EMT) by SOCS1 in colon cancer cell lines (9-11).

On the contrary, anti-apoptotic and pro-tumor effects of SOCS have been also reported (12-14). In Jurkat T cells, TNF alpha-induced or ROS-mediated apoptosis can be inhibited by SOCS1 over-expression through attenuation of Jak1 with protection of protein tyrosine phosphatases (12). There is a positive correlation of cell growth or resistance to cell death with SOCS1 expression in colon cancer cells (13). Silencing SOCS1 can inhibit subcutaneous tumor growth of melanoma cells and metastatic development in the lung (14).

With these differential effects of SOCS in diverse tumor cell systems reported, the ability of SOCS to affect tumor sensitivity to therapeutic modality such as ionizing radiation (IR) has been of interest. In this respect, SOCS3 has exhibited opposite effects on radiosensitivity of cells in glioblastoma multiforme (GBM) and non-small cell lung

carcinoma (NSCLC) by activating Erk/MAPK and blocking entry into S phase, respectively (15, 16). Sitko et al. (17) have observed that SOCS3-deficient MEF cells are accumulated in the G2/M phase without undergoing G1 phase arrest upon irradiation. G1 arrest was resumed by re-introducing SOCS3 gene accompanied by p21 up-regulation in the absence of p53 activation (17). On the other hand, much less is known for the effect of SOCS1 on radiation response through the regulation of cell cycle, apoptosis, and survival affecting tumor growth.

We have utilized colorectal cancer (CRC) cells constructed for over-expression or knock-down of SOCS1 to investigate its anti-tumor action through the regulation of radiation response. In the present work we found that SOCS1 promotes radiosensitivity of HCT116 CRC cells to apoptosis by counteracting radiation signals for survival mediated by Jak/Erk and STAT3 and promoting p53 activity. This was accompanied by a shift of cell cycle arrest from G2/M to G1. Importantly, we have noted that radiation-induced early ROS generation was responsible for the survival signal to induce cell cycle progression, which is subject to counter-regulation by SOCS1. Such radiosensitizing actions of SOCS1 might be useful for developing anti-tumor regimens to overcome resistance to radiation therapy, particularly for tumors with low levels of SOCS1 expression.

II. Results

1. SOCS1 promotes gamma irradiation-induced apoptosis of colorectal cancer cells with increases of DNA damage and p53 activation

Previous studies have demonstrated the apoptosis-regulatory function of SOCS can differ depending on the apoptosis-stimuli and target cell systems employed (5-10). In order to study the role of SOCS in IR-induced apoptosis, we utilized HCT116 colorectal cancer (CRC) cells The HCT116 cell line was chosen as the target cell system upon screening a panel of colorectal cancer cell lines since it showed moderate expression levels of SOCS1, making it suitable for both over-expression and knock-down for SOCS1 (10).

HCT116 cells bearing wild-type functional p53 gene (18), denoted as HCT116/p53wt, were exposed to varying doses of γ-IR. Apoptosis was then measured. A modest induction of apoptosis was observed at 24 h after radiation in a dose-dependent manner (130 ~ 160 % increase in mean fluorescence intensity (MFI) over control upon exposure to 5 ~ 20 Gy, Fig. 1A). Compared to mock cells, SOCS1-transduced cells exhibited generally enhanced apoptosis upon IR treatment. Radiation-induced DNA damage that led to apoptosis was then assessed using TUNEL assays. Results indicated that DNA cleavage was progressively increased by 48 h in parental HCT116/p53 wt cells, which was further enhanced in SOCS1-transduced cells (Fig. 1B). Accordingly, apoptosis determined at 48 h revealed 3-fold increase in Annexin-V positive populations, which was further enhanced in SOCS1 over-expressing cells (Fig. 1C). Apoptosis was significantly increased (45 % increase) in SOCS1-transduced cells but significantly decreased in SOCS1 knock-down cells (40 % decrease) as compared to that in respective mock cells (Fig. 1C), showing the pro-apoptotic effect of SOCS1. As a mediator of apoptotic response, p-p53 level representing p53 activation was increased by γ-

IR. It was further enhanced in SOCS1 over-expressing cells as compared to mock cells (Fig. 1D). On the other hand, p-p53 level appeared to be reduced in SOCS1-ablated cells. The regulation profile of apoptosis resembled that of p53 activation in SOCS1-overexpressing and SOCS1-ablated cells, indicating that apoptosis promotion by SOCS1 was associated with p53 activation (Figs. 1C and 1D).

2. Modulation of IR-induced cell cycle arrest by SOCS1 from G2/M to G1

To investigate the underlying mechanism for SOCS1-mediated promotion of IR-induced apoptosis, the effect of SOCS1 on radiation-induced cell cycle changes was analyzed. Upon exposure to γ-IR at 5 Gy, HCT116/p53wt cells exhibited a prominent G2/M phase arrest with decreases of G1 and S phase cells. It was noted that SOCS1 over-expressing cells upon radiation displayed a significant increase in G1 arrest and a suppression in G2/M arrest as compared to mock cells (Fig. 2A). In contrast, SOCS1 knock-down caused a decrease in G1 arrest and an increase in G2/M arrest (Fig. 2B). Such effect was correlated with changes in expression levels of p21 and cyclin B. Over-expression of SOCS1 induced up-regulation of p21 and down-regulation of cyclin B at 24 h after γ-IR (Fig. 2C), whereas SOCS1 knock-down caused the opposite regulation (Fig. 2D). These data strongly indicate that SOCS1 can regulate γ-IR-induced cell cycle changes, leading to the promotion of G1 arrest and subsequent steps to p53-mediated pro-apoptotic response. Instead, G2/M population was reduced with a decrease in cyclin B, a G2/M cyclin in HA-SOCS1 cells.

We noted that SOCS1 expression was substantially induced within 1-4 h post irradiation, suggesting a potential regulatory role of SOCS1 during radiation-induced response in these cells (Fig. S1-A, Fig. 1D). Unlike SOCS1, SOCS3 over-expression had no

effect on cell cycle regulation or apoptosis induced by γ-IR in these cells, consistent with the lack of SOCS3 induction by γ-IR (Fig. S1). We also noted that p53-null HCT116 cells did not show notable changes in cell cycle or apoptotic response upon SOCS1 over-expression, suggesting a p53-dependent apoptosis-promoting effect of SOCS1 (Fig. S2), which might be associated with p21-mediated G1 arrest. The radiosensitizing effect of SOCS1 on apoptosis was also observed in RKO, another human colon cancer cell line bearing wt p53 (Fig. S3), but not in Jurkat T cells with p53 mutation (Fig. S3-C). In fact, an opposite regulatory effect of SOCS1 causing suppression of γ-IR-induced apoptosis in Jurkat T cells was associated with an increase in cells at G2/M arrest (Fig. S3-D).

3. SOCS1 targets γ-IR-induced activation of Jak3, Erk, and STAT3 to induce G1 arrest and suppress cell cycle progression

Prior to undergoing apoptosis upon receiving radiation, tumor cells may activate survival signaling pathways to cope with radiation stress. In fact, HCT116 cells exposed to γ-IR at an apoptosis-inducing dose of 5 Gy exhibited early activation of Jak/STAT and MAPK family members including Jak1/Jak3, STAT1/STAT3, and Erk/p38 within 15 min to 1 h (Fig. 3-A). To identify signaling pathways affected by SOCS1 during the radiation response, effects of SOCS1 over-expression or knock-down on these factors were examined. Results showed that Jak3, STAT3, and Erk activities induced by irradiation were generally down-regulated in SOCS1 over-expressing cells (Fig. 3-A), while these were up-regulated in SOCS1-ablated cells at basal or IR-induced levels (Fig. S4). Other Jak/STAT and MAPK family members examined were either not notably activated upon γ-IR (e.g., Jak2, STAT5, STAT6) or activated upon γ-IR treatment but not regulated by SOCS1 (e.g., Jak1, STAT1, p38,

Jnk). These results suggest that Jak3/STAT3 and Erk could be potential targets of SOCS1 action in γ-IR-induced stress response in HCT116 cells, which might lead to cell cycle arrest at G1 phase and subsequent apoptosis. To explore the signaling mechanism of SOCS1 action, we examined effects of specific inhibitors for Jak/STAT and Erk pathways on the IR-induced response regulated by SOCS1.

.Elevated levels of both phospho-Erk and phospho-STAT3 in shSOCS1 cells were down-regulated in the presence of AG490, a Jak inhibitor (Fig. S4 and Fig. 3B). However, levels of phospho-STAT3, but not phospho-Jak3, were suppressed upon treatment with an MEK/Erk inhibitor PD98059 (Fig. 3C). This indicates that Jak3 can control Erk and subsequently regulate STAT3 activities during the radiation response as targets of SOCS1. In addition, it was noted that the reduction in G1 arrest in SOCS1-ablated cells was substantially restored by treatment with AG490 and PD98059 with up-regulated p21 expression. These data indicate that suppression of pro-survival pathways by SOCS1 can interfere with cell cycle progression from G1, which can act in concert with the promotion of p53-dependent pro-apoptotic response under radiation.

4. Role of ROS signal in γ-IR-induced cell survival pathways which is counteracted by SOCS1

Our earlier studies have demonstrated that SOCS1 can counteract ROS signals and regulate cell survival and apoptosis under oxidative stress (10, 12). We have also shown that exposure to hydrogen peroxide or low dose γ-IR given by fractionized ionizing radiation can induce ROS-mediated activation of EMT signaling and that SOCS1 can down-regulate ROS levels to suppress EMT response involving Src/Akt/ Erk in HCT116/p53wt cells (10, 11).

Having observed early activations of Jak3, Erk, and STAT3 upon receiving apoptosis-inducing dose of γ-IR and their suppressions by SOCS1, we examined the role of ROS signal during IR-induced stress response and the potential role of SOCS1 in ROS regulation. When HCT116 cells were exposed to γ-IR (5 Gy), Erk and STAT3 showed modest activation along with induction of SOCS1 in 30 min, all of which were down-regulated by a pretreatment with N-acetyl cysteine (NAC), an antioxidant (Fig. 4-A). Indeed, NAC-sensitive ROS generation was observed under this condition (Fig. 4-B). NAC treatment not only suppressed early activation of Erk and STAT3, but also induced a significant increase in apoptotic cell populations by 24 h, suggesting a role of ROS in mediating cell survival pathways (Fig. 4C). Importantly, under apoptosis condition, ROS levels were suppressed in SOCS1-transfected cells, while early ROS generation were increased in SOCS1 knock-down cells (Figs. 4D and 4E). Upon thioredoxin (Trx1) over-expression, cells displayed impaired response to γ-IR for early ROS generation and increased apoptotic response by 24 h (Figs. 4F and 4G). These results suggest that the ROS signal is responsible for the early phase activation of survival signaling pathways triggered by γ-IR and that SOCS1 down-regulation of ROS levels may interfere with this process, contributing to increased apoptotic response to radiation.

III. Discussion

Radiation therapy is a major anti-cancer regimen. Ionizing radiation is known to exert tumor killing effects by causing DNA damage with double-strand breaks. IR induces mitotic arrest directly and by generating high levels of ROS indirectly which leads to DNA damage (19). In this regard, resistance to radiation therapy is often correlated with low ROS-inducing potential of tumor cells. In fact, sublethal doses of ionizing radiation can generate low ROS levels which may trigger survival signaling pathways (19). Thus, to increase tumor sensitivity to radiation, not only the activation of DNA damage leading to apoptosis, but also the suppression of cell survival signaling through ROS regulation should be considered.

Although SOCS1 has emerged as a potent tumor suppressor in various cancers, its mechanism of action in the regulation of tumor sensitivity to radiation remains unclear. Thus, the present study was conducted to investigate radiosensitizing effects of SOCS1 using a colorectal cancer model. Our data indicated that while γ-IR induced ROS mediating Jak/STAT3 and Erk activation for cell survival signaling, SOCS1 counteracted these pathways by down-regulating ROS. This result is in line with our recent studies showing that SOCS1 can also suppress the invasion and EMT induced by hydrogen peroxide and fractionated ionizing radiation via ROS regulation in colorectal cancer cells (10, 11).

Induction of thioredoxin (Trx1) has been found to be responsible for SOCS1-mediated ROS down-regulation in colon cancer cells (10, 11). Thus, we examined the role of Trx1 in cell survival vs. apoptotic response induced by radiation. Indeed, Trx1 transfection caused a blockade in the early increase of ROS level and promoted the apoptotic response induced by radiation, supporting the role of ROS signal in SOCS1-mediated cell survival under radiation stress.

SOCS1 and SOCS3 share common structural features such as SH2 domain, Jak kinase inhibitory domain, and SOCS-box motif. However, regulatory effects of SOCS1 and SOCS3 on radiation-induced DNA damage response and apoptosis seem to differ depending on cell types (15, 16). We have observed that while SOCS1 exhibited a pro-apoptotic function in p53 wt /HCT116 CRC cells with cell cycle shift to G1, SOCS3 had no effects on the apoptosis or cell cycle changes induced by γ-IR (Fig S1). In p53 wt-bearing RKO CRC cells, however, both SOCS1 and SOCS3 promoted apoptotic response (Fig S3-A and S3-B).

As we observed p53 activation in HCT116 CRC cells, p53-dependent p21 induction and inhibition of cell cycle progression from G1 to S phase by SOCS1-mediated attenuation of Jak3/Erk/STAT3 might have resulted in accumulation of cells in G1 arrest when cells became apoptotic in time. This result was in a good agreement with the role of Jak3/STAT3 inhibition in the induction of cell cycle arrest and apoptosis in colon carcinoma cells (20). STAT3 has been implicated in the promotion of cell survival and cell cycle progression as well as in the stimulation of tumor metastasis through MMP7 induction (21, 22). Thus, SOCS1-mediated JAK/STAT3 pathway inhibition might have contributed to overall anti-tumor and anti-metastatic effects of SOCS1 on colon cancers. Together, these results support the anti-invasive and anti-EMT role of SOCS1 through Jak/STAT inhibition in colon cancer models (10, 22).

The induction of G1 arrest and pro-apoptotic function of SOCS1 appeared to be p53-dependent as p53-null cells were unresponsive to SOCS1 over-expression (Fig. S2). In addition, Jurkat (p53 mt) cells showed anti-apoptotic response with increased G2/M arrest upon SOCS1 over-expression (Figs. S3-C and S3-D). In this regard, the lack of significant apoptosis noted in HCT116/p53 null cells seemed to correlate with the increased G2/M arrest

upon radiation as compared to p53 wt cells (Fig. S2, 23). As the ability of SOCS1 to activate p53 through the interaction with ATM has been suggested (24), it is likely that SOCS1 induced by γ-IR participates in DNA damage response in a positive feed-back loop to modulate cell cycle changes and to increase radiosensitivity for apoptosis. In summary, data of the present study suggest that radiosensitizing anti-tumor action of SOCS1 is exerted though suppression of ROS-mediated survival signaling leading to cell cycle arrest at G1 and promotion of apoptotic response in colon cancer cells (Fig S5). As the emergence of radioresistance in diverse tumor cells poses difficulty in radiation therapy, SOCS1 might be considered as a radiosensitizing agent not only through modulation of DNA damage response and cell cycle arrest, but also through regulation of γ-IR-induced ROS and associated survival signaling pathways in relevant tumor models.

IV. Materials and Methods

1. Cell culture and gene transfection

Cell culture conditions and details for gene transfection to obtain stable cell lines are described in Supplemental materials.

2. Radiation treatments of cells

Cells were irradiated with 2 ~ 20 Gy at room temperature with a ^{137}Cs γ-source irradiator at a dose rate of 5.66 Gy/min using an IBL 437 type H irradiator (CIS Biointernational, Nice, France) (11).

3. Apoptosis measurement and cell cycle analysis

Methods used for the analysis of apoptotic populations and cell cycle were provided in Supplemental materials.

4. Western blot and densitometric analysis

Immunoblotting with respective antibodies as well as the densitomertic analysis are described in Supplemental materials.

5. Terminal deoxynucleotide transferase dUTP nick end labelling (TUNEL) assays

Methods used for TUNEL assays were provided in Supplemental materials.

6. ROS measurement

Intracellular ROS levels were determined as described previously (11).

7. Statistical analysis

For statistical analysis, the experiments were performed at least in three independent sets. The values are presented as means ± SE. Statistical significance was determined by a Student's t-test. A value of * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant.

Figure Legends

Fig. 1. SOCS1 promotes radiation-induced apoptosis of HCT116 cells involving increased DNA damage and p53 activation. HCT116/p53wt cells stably transfected with HA and HA-SOCS1 constructs were exposed to indicated doses of γ-IR. Cells were harvested at 24 h and apoptotic cells were measured by Annexin-V staining (A). Cells were treated with γ-IR at 5 Gy (B, C, D) and analyzed for DNA damage by performing Terminal deoxynucleotide transferase dUTP nick end labelling (TUNEL) assays (B). Apoptosis was determined at 48 h upon receiving 5 Gy (C). Western blot was performed for p-p53, p53, and SOCS1 with densitometric analysis of protein levels (D).

Fig. 2. **Modulation of radiation-induced cell cycle arrest by SOCS1, reducing IR-induced G2/M arrest and promoting G1 arrest.** Cell cycle analysis of HCT116/p53 wt cells stably transfected with HA and HA-SOCS1 or sh and shSOCS1 were performed at 24 h post irradiation of 5 Gy by flow cytometry using the Cellquest program as described in the text (A and B). Cells were subjected to immunoblotting to analyze expression levels of cell cycle marker proteins (C and D: Fig. S6-A).

Fig. 3. Radiation-induced early activation of Jak3/STAT3 and Erk is suppressed by SOCS1, and the inhibition of Jak3/STAT3 and Erk in shSOCS1 cells leads to G1 arrest restoration. Analysis of Jak/STAT and MAPK activation kinetics induced by γ-IR. HCT116/p53 wt cells stably transfected with HA and HA-SOCS1 were exposed to γ-IR at 5 Gy and harvested at indicated time points. Cell lysates were prepared to analyze Jak/STAT and MAPK activation status by immunblotting (A). sh and shSOCS1-transfected cells were analyzed for Jak/STAT and MAPK activation status and cell cycle changes upon irradiation with or without pre-treatment with inhibitors of Jak (AG490) or Erk (PD). Effects of AG490 and PD on on Jak/STAT and Erk were examined (B and C: Fig. S6-B).

Fig. 4. SOCS1 counter-regulates reactive oxygen species (ROS) responsible for the survival signal during radiation response to promote apoptosis. HCT116 /p53 wt cells were irradiated with γ-IR at 5 Gy with or without NAC treatment (1 h pretreatment at 1mM). Cells were analyzed for signaling mediators by immunoblotting (A) and ROS generation (B) at 30 min. Cell death was measured at 24 h post irradiation by propidium iodide and Annexin-V staining (C). The γ-IR-induced ROS generation was assessed in cells with SOCS1 knock-down at 10 min and with SOCS1 over-expression at indicated time points (D and E). HCT116 /p53 wt cells transfected with Flag or Flag-Trx1 were irradiated at 5 Gy and analyzed for ROS generation, apoptotic response and Trx1 expression (F and G).

V. References

1.Yoshimura A, Naka T and Kubo M (2007) SOCS proteins, cytokine signaling and immune regulation. Nat Rev Immunol. 7, 454-465.

2. Sutherland KD, Lindeman GJ, Choong DY et al. (2004) Differential hypermethylation of SOCS genes in ovarian and breast carcinomas. Oncogene. 23, 7726-7733.

3. Yoshikawa H, Matsubara K, Qian GS et al (2001) SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat Genet 28, 29-35.

4 Oshimo Y, Kuraoka K, Nakayama H et al (2004) Epigenetic inactivation of SOCS-1 by CpG island hypermethylation in human gastric carcinoma. Int J Cancer 112, 1003-1009.

5. Oh J, Kim SH, Ahn S and Lee CE. Suppressors of cytokine signaling promote Fas-induced apoptosis through down-regulation of NF-κB and mitochondrial Bfl-1 in leukemic T cells. J Immunol 189, 5561-5571.

6. Neuwirt H, Puhr M, Santer FR et al (2009) Suppressor of cytokine signaling (SOCS)-1 is expressed in human prostate cancer and exerts growth-inhibitory function through down-regulation of cyclins and cyclin-dependent kinases. Am J Pathol 174, 31921-31930.

7. Neuwirt H, Puhr M, Cavarretta IT et al (2007): Suppressor of cytokine signalling-3 is up-regulated by androgen in prostate cancer cell lines and inhibits androgen-mediated proliferation and secretion. Endocr Relat Cancer 14, 1007-1019.

8. Scutti JA, Matsuo AL, Pereira FV et al (2011). Role of SOCS-1 gene on melanoma cell growth and tumor development. Transl Oncol 4, 101-109.

9. David M, Naudin C, Letourneur M et al (2014) Suppressor of cytokine signaling 1 modulates invasion and metastatic potential of colorectal cancer cells. Mol Oncol. 8, 942-955.

10. Jung SH, Kim SM and Lee CE (2016) Mechanism of suppressors of cytokine signaling inhibition of epithelial-mesenchymal transition signaling through ROS regulation in colon cancer cells: suppression of Src leading to thioredoxin up-regulation. Oncotarget 7, 62559-62571.

11 Kim S, Kim SH and Lee CE (2020) SOCS1 represses fractionated ionizing radiation-induced EMT signaling pathways through the counter-regulation of ROS scavenging and ROS-generating systems. Cell Physiol Biochem 54, 1026-1040.

12. Oh J, Hur MW and Lee CE (2009) SOCS1 protects protein tyrosine phosphatases by thioredoxin up-regulation and attenuates JAKs to suppress ROS-mediated apoptosis. Oncogene. 28, 3145-3156.

13. Tobelaim WS, Beaurivage C et al (2015) Tumour-promoting role of SOCS1 in colorectal cancer cells. Sci Rep 5, 14301.

14. Huang FJ, Steeg PS, Price JE et al (2008) Molecular basis for the critical role of suppressor of cytokine signaling-1 in melanoma brain metastasis. Cancer Res 68, 9634-9642

15. Zhou H, Miki R, Eeva M et al (2007) Reciprocal regulation of SOCS1 and SOCS3 enhances resistance to ionizing radiation in glioblastoma multiforme. Clin Cancer Res 15, 2344-2353.

16. Lin YC, Lin CK, Tsai YH et al (2010) Adenovirus-mediated SOCS3 gene transfer inhibits the growth and enhances the radiosensitivity of human non-small cell lung cancer cells. Oncol Rep 24, 1605-1612.

17. Sitko JC, Yeh B, Kim M et al (2008) SOCS3 regulates p21 expression and cell cycle arrest in response to DNA damage. Cell Signal 20, 2221-2230.

18. Liu Y and Bodmer WF (2006) Analysis of P53 mutations and their expression in 56 colorectal cancer cell lines, Proc Natl Acd Sci 103, 976-981.

19. Perillo B, Donato MD, Pezone A et al (2020) ROS in cancer therapy: The bright side of the moon. Exp Mol Med 52, 192-302.

20. Lin Q, Lai R, Chirieac LR et al (2005) Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: Inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. Am J Pathol 167, 969-980.

21. Sugase T, Takahashi T, Serada S et al (2017) SOCS1 gene therapy improves radiosensitivity and enhances irradiation-induced DNA damage in esophageal squamous cell carcinoma. Cancer Res 7, 6975-6986.

22. Xiong H, Zhang ZG, Tian XQ et al (2008) Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. Neoplasia 10, 287-297.

23. Merritt AJ, Allen TD, Potten CS and Hickman JA (1997) Apoptosis in small intestinal epithelial from p53-null mice: evidence for a delayed, p53-independent G2/M-associated cell death after gamma-irradiation. Oncogene 14, 2759-2766.

24. Calabrese V, Mallette FA, Deschênes-Simard X et al (2009) SOCS1 links cytokine signaling to p53 and senescence. Mol Cell 11, 754-767.

BMB Reports: 21-191

Main Figures

SOCS1 counter-acts the ROS-mediated survival signals and promotes apoptosis with cell cycle modulation to increase radiosensitivity of colorectal cancer cells

Ji-Yoon Ryu, Jiyoung Oh, Su-Min Kim, Won-Gi Kim, Hana Jeong, Shin-Ae Ahn, Seol-Hee Kim, Byong Chul Yoo and Choong-Eun Lee*

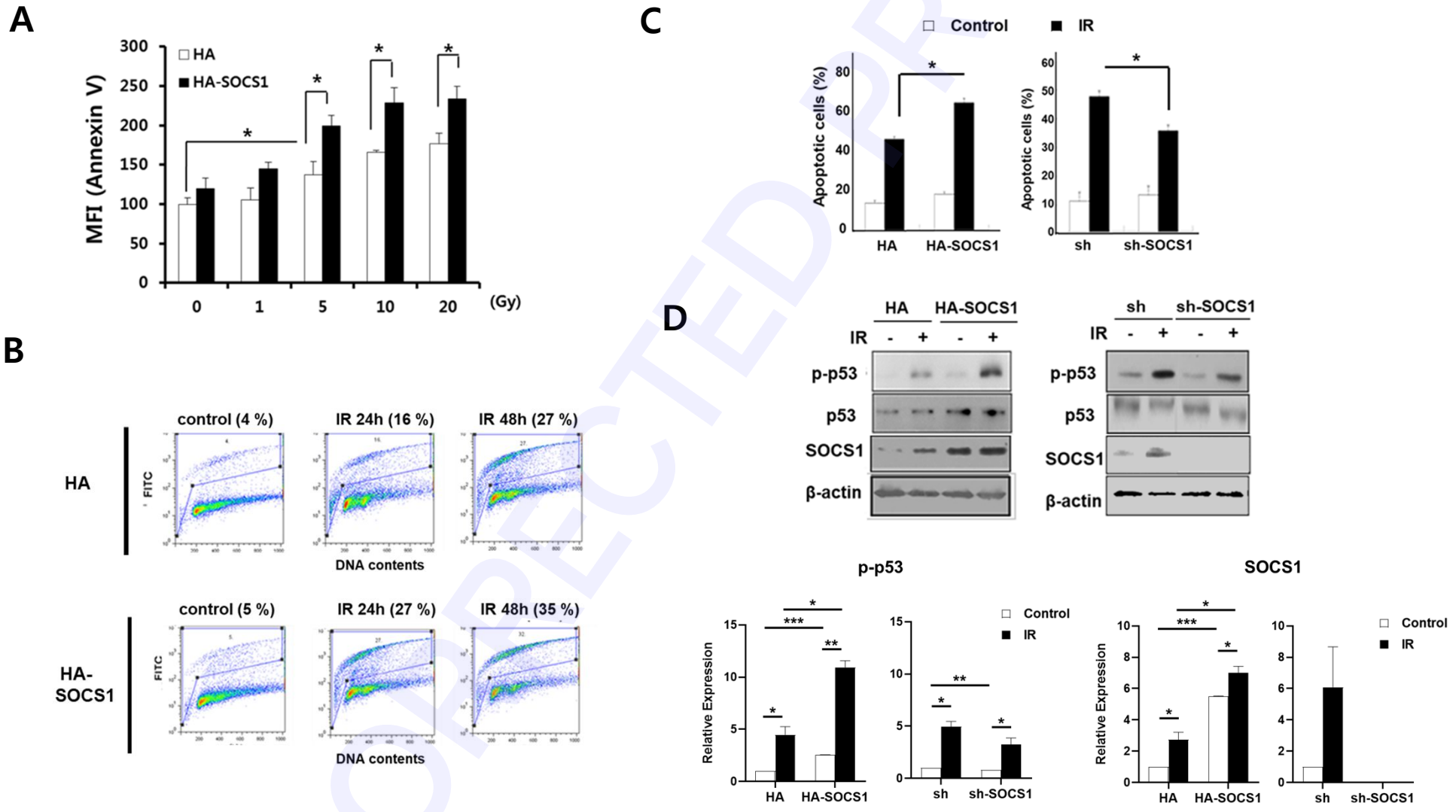

Fig 1. SOCS1 promotes IR-induced apoptosis of HCT116 cells involving increased DNA damage and p53 activation.

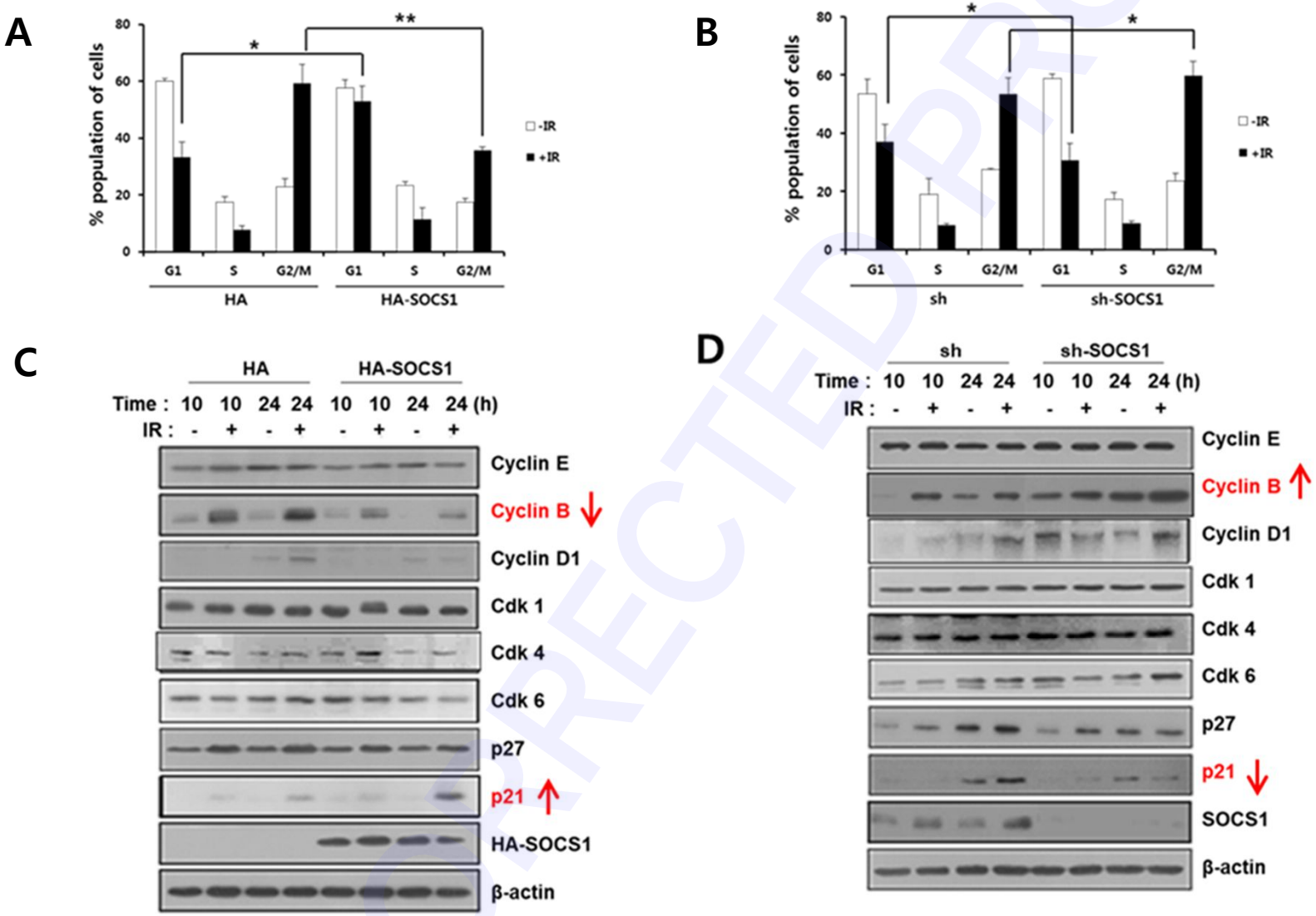

Fig 2. Modulation of IR-induced cell cycle arrest by SOCS1: SOCS1 reduces *IR-induced G2/M arrest and promotes* G1 arrest with counter-regulation of associated markers cyclin B and p21, respectively.

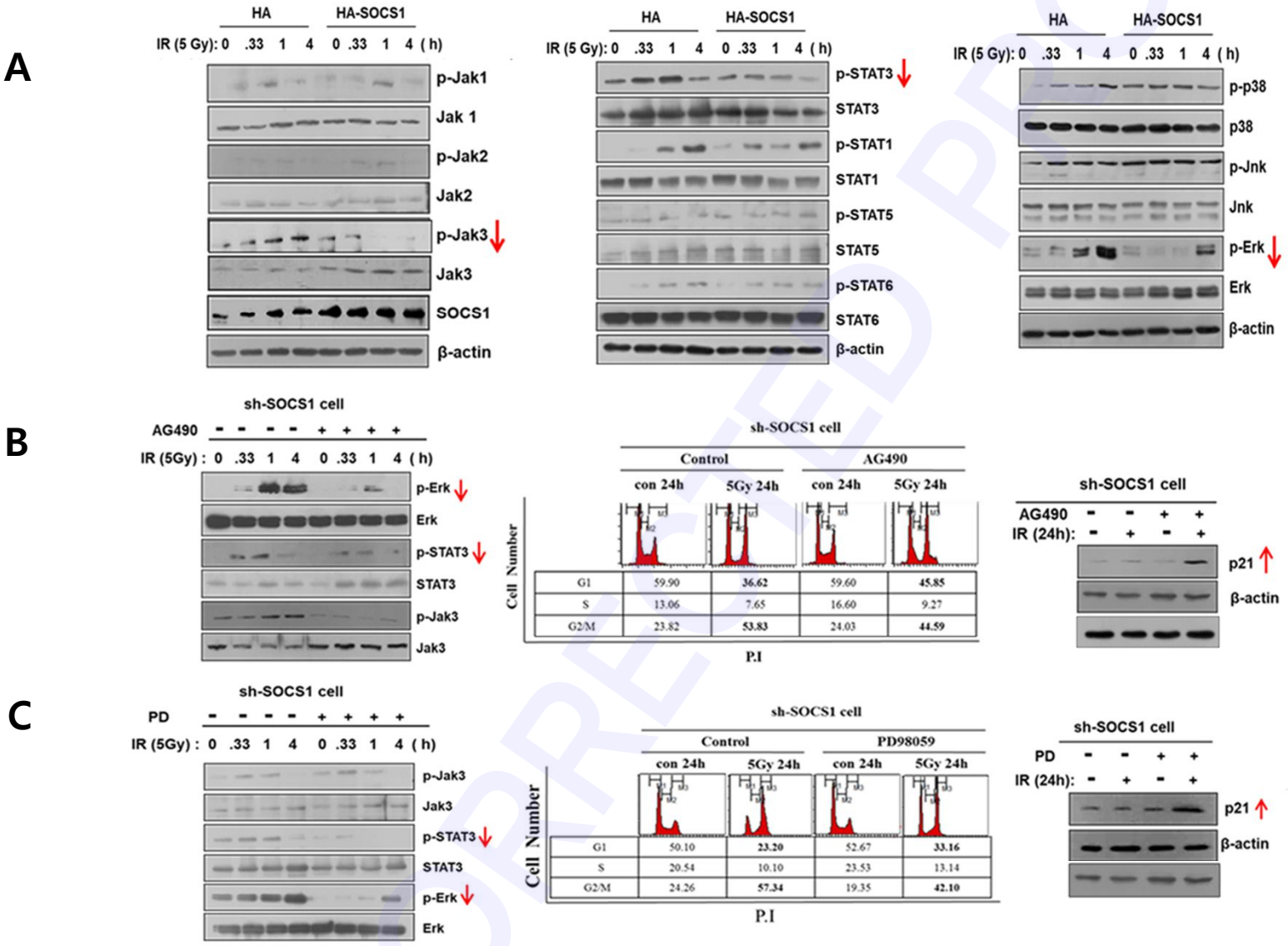

Fig 3. IR-induced early activation of Jak3/STAT3 and Erk is suppressed by SOCS1 and the inhibition of the IR-induced activation of Jak3 and Erk in shSOCS1 cells leads to G1 arrest restoration.

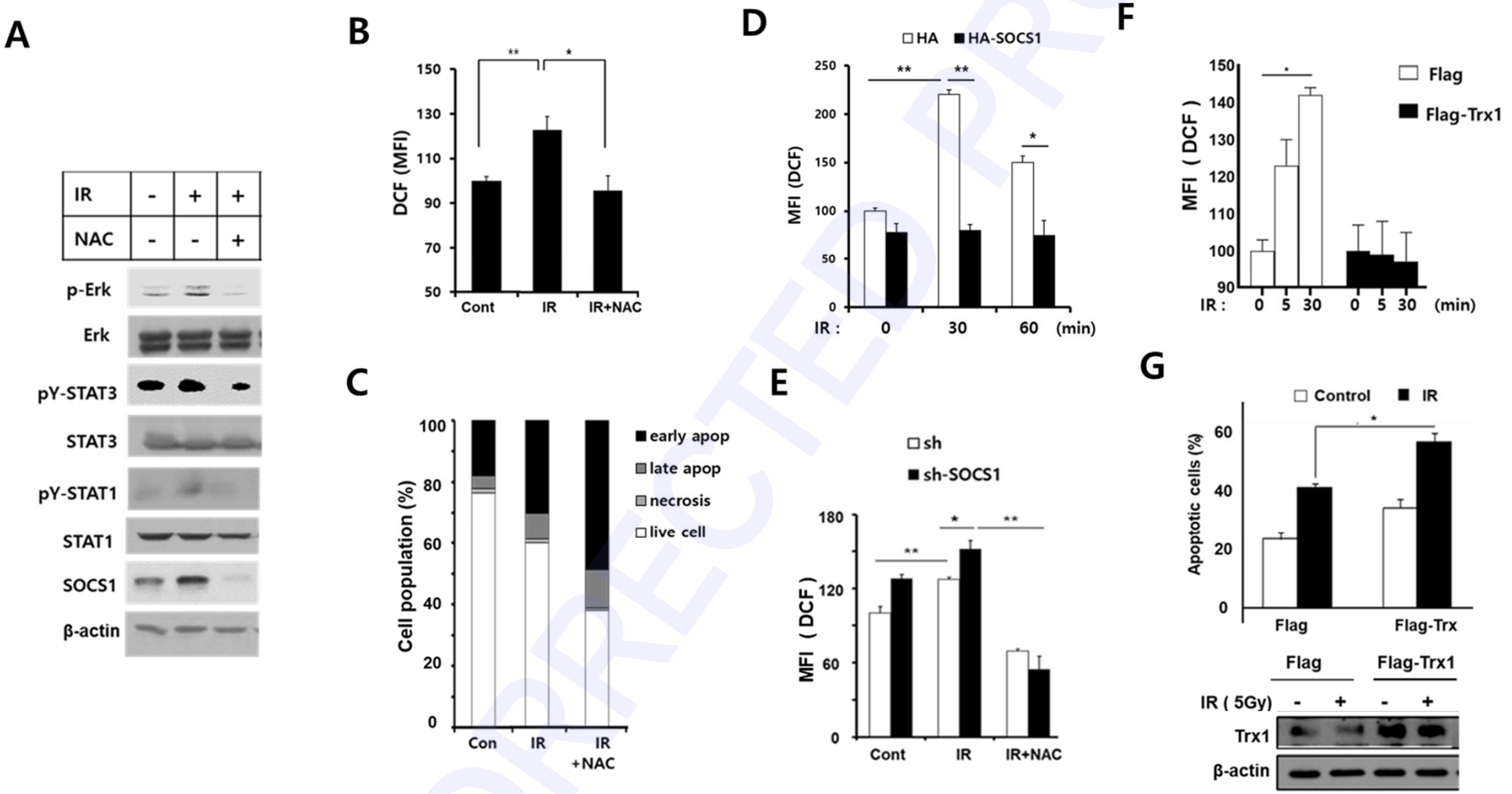

Fig 4: SOCS1 counter-acts early ROS upregulation responsible for the survival signal during radiation response leading to apoptosis.

BMB Reports: 21-191

< Supplementary Materials >

SOCS1 counter-acts on the ROS-mediated survival signals and promotes apoptosis with cell cycle modulation to increase radiosensitivity of colorectal cancer cells

< Materials Methods >

Cell culture

The human colorectal cancer cell (CRC) lines (HCT116/p53 wt, HCT116/p53 null, RKO) and leukemic Jurkat T cells were maintained in DMEM and RPMI media containing 10 % fetal bovine serum (GIBCO), respectively. Cells were cultured in a humidified 5% CO_2 incubator at 37℃.

Gene transfection

Cells ($1x10^6$) were mixed with 5 μg of each of pcDNA-HA, HA-SOCS1, non-targeting shRNA, shSOCS1 and Flag-Trx1 constructs as described (11,12). pcDNA-Myc and Myc-SOCS3 constructs (5) were also used for transfection. The cell mixture was transferred into a 0.4 cm electrode gap cuvette (Bio-Rad Laboratories) and subjected to 5 pulses of 250 V for 5 ms using a Gene Pulser X cell electroporation system (Bio-Rad). Transfected cells were cultured in the selection media containing G418 for HA and HA-SOCS1 cells or puromycin for sh and shSOCS1 cells.

Apoptosis measurement

Cell death determination was conducted by Annexin V and PI staining as described (5). Both early apoptotic (Annexin V-positive/ PI-negative) and late apoptotic (Annexin V-positive/ PI-positive) cells were included in cell death determinations, unless otherwise stated. The stained cells were analyzed using a FACS Calibur flow cytometry system (BD Bioscience).

Cell cycle analysis

CRC cells treated with or without gamma irradiation, were harvested at the indicated times by trypsinization, washed with PBS, and pelleted by centrifugation. Cells were stained with PI, and immediately analyzed by flow cytometry using Cellquest software (BD Bioscience).

TUNEL assays

TUNEL analysis was conducted with using a commercial APO-BRDU kit (Phoenix Flow Systems). Briefly, harvested cells (1×10^6) were fixed and labeled with TdT and BrdUTP solution for 60 min. Then cells were incubated with FITC-anti-BrdU antibodies after which PI/RNase A staining solution was added. The stained cells were analyzed using a FACSCalibur flow cytometry system.

Antibodies used for immunoblotting

The following primary antibodies were used: anti-HA, anti-Erk, anti-pY/pS-Erk, anti-p38, anti-p-p38, anti-Jnk, anti-p-Jnk, anti-Jak1, anti-p-Jak1, anti-Jak2, anti-p-Jak2, anti-Jak3, anti-p-Jak3, anti-STAT1, anti-pY-STAT1, anti-STAT3, anti-pY-STAT3, anti-STAT5, anti-pY-STAT5, anti-STAT6, anti-pY-STAT6, and anti-β-actin antibodies from Cell Signaling Technologies ; anti-SOCS1, anti-p21, anti-p27, anti-p53, anti-p-p53, anti-Bax, anti-cyclin A, anti-cyclin B, anti-cyclin D1, anti-cyclin E, and anti-SOCS3 antibodies from Santa Cruz Biotechnology. The immunoblots were revealed by incubation with HRP-conjugated anti-mouse, anti-rabbit or anti-rat secondary Abs (Cell Signaling Technologies and Santa Cruz Biotechnology) and subjected for detection using an ECL system (Amersham).

Densitometric Analysis

Western blot data were subject to densitometric analysis to determine relative expression levels of proteins using ImageJ software as described (10). The protein expression levels quantified are compiled and shown in Fig S6. Relative expression is shown as the expression ratio determined from the band intensity of the respective protein over that of beta-actin for the untreated control taken as 1. Results show data (mean + SE) obtained from multiple blots.

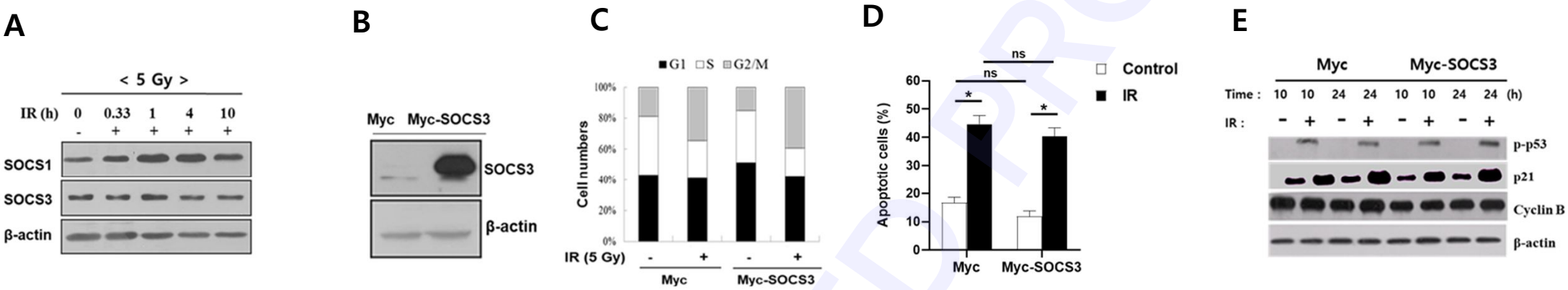

Fig S1. SOCS1 but not SOCS3 is induced in response to the IR (A) and SOCS3 over-expression does not affect the IR-induced apoptosis and cell cycle changes. HCT116/p53 wt cells were treated with γ-IR at 5 Gy and the expression levels of SOCS1 and SOCS3 were analyzed by Western blotting (A). SOCS3 over-expressing cells were constructed (B) and analyzed for the γ-IR-induced cell cycle changes at 24 h (C) and apoptosis at 48 h (D). Effects of SOCS3 on the expression levels of cell cycle markers and apoptosis in response to γ-IR were also analyzed (E).

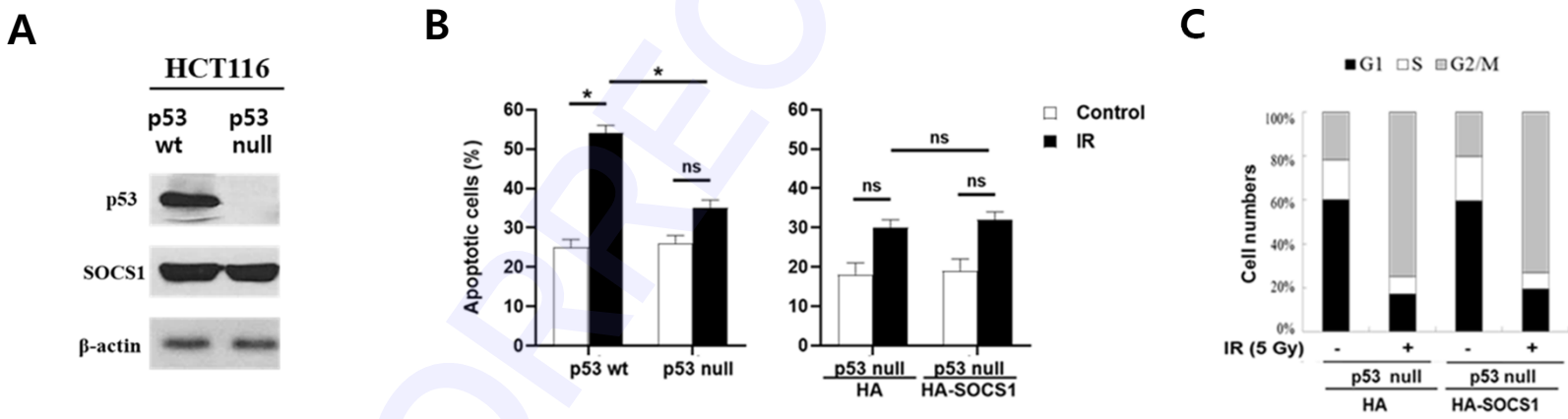

Fig S2. Apoptosis-promoting and cell cycle-modulating effects of SOCS1 is p53-dependent in HCT 116 CRC cells HCT116 /p53 wt and HCT116 /p53 null cells (A) were treated with γ-IR at 5 Gy and analyzed for apoptotic response along with HCT116 /p53 null cells stably transfected with HA or HA-SOCS1 at 48 h (B) and cell cycle changes at 24 h (C).

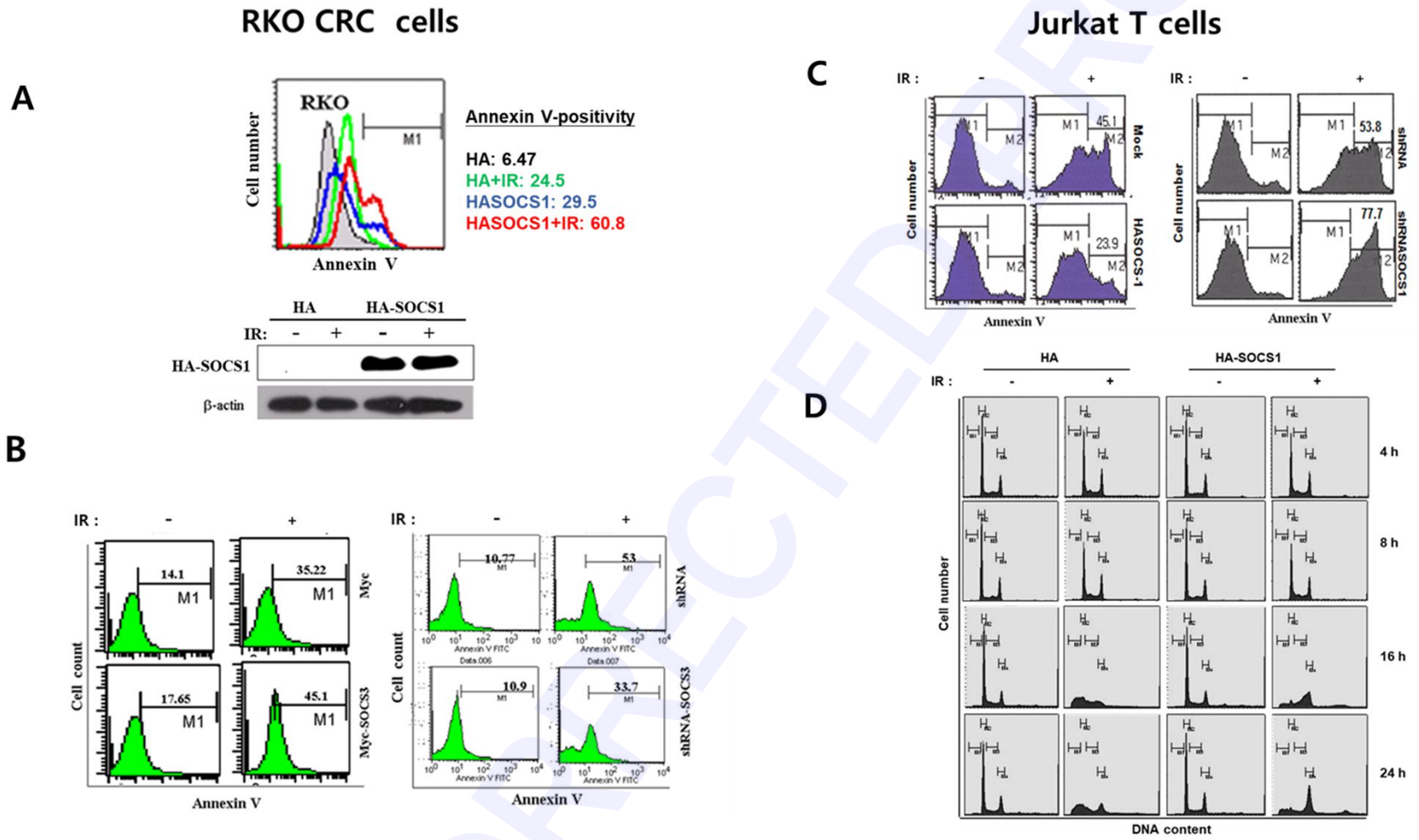

Fig S3. SOCS1 promotes radiation-induced apoptosis in RKO (p53 wt) CRC cells, but exhibits opposite effects in Jurkat T (p53 mt) cells with increased G2/M arrest. RKO colorectal cancer cells transfected with HA and HA-SOCS1 (A) or SOCS3 vs shSOCS3 (B) received γ-IR for 24 h at 50 Gy to induce apoptosis which was determined by Annexin-V staining. Jurkat leukemic T cells transfected with SOCS1 vs shSOCS1 were analyzed for apoptosis in response to γ-IR at 20 Gy (C). The HA and HA-SOCS1 transfected Jurkat T cells were analyzed for the IR-induced cell cycle changes by 24 h (D).

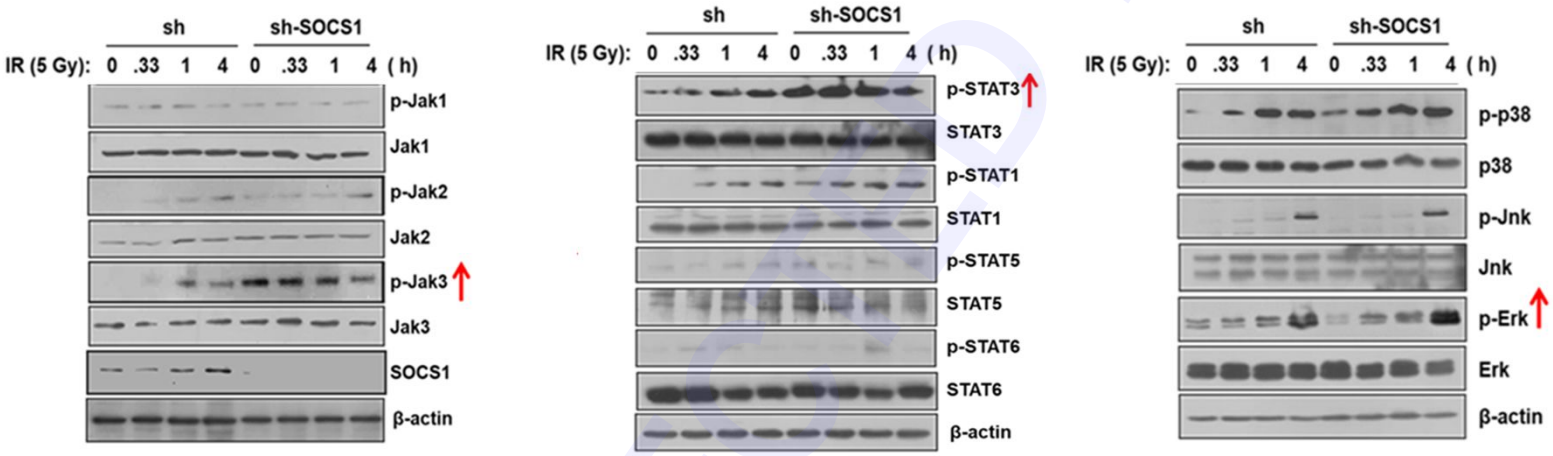

Fig S4. Analysis of Jak/STAT and MAPK activation profile induced in response to IR in sh vs shSOCS1 cells. HCT116/p53 wt cells stably transfected with sh and shSOCS1 were analyzed for Jak/STAT and MAPK activation kinetics induced by γ-IR by immunoblotting.

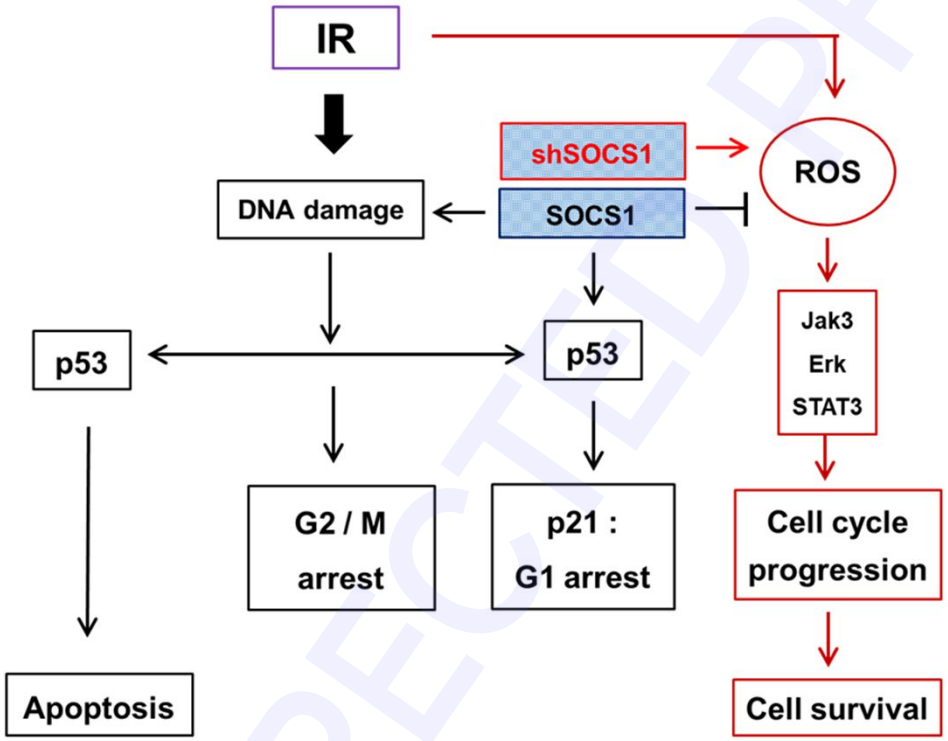

Fig S5. Schematic diagram for the regulation of the IR-induced response by SOCS1 to increase radiosensitivity of colorectal cancer cells by suppressing ROS-mediated survival pathway (red line) and promoting cell death pathway with cell cycle modulation (black line).
In addition to induce direct DNA damage, ionizing radiation triggers the generation of modest levels of intracellular ROS in the early phase which can activate survival signals through the activation of Jak3/Erk/STAT3. SOCS1 counter-acts on the survival pathway by down-regulation of ROS, which results in the inhibition of cell cycle progression from G1 to S, thereby inducing cells in G1 arrest. SOCS1 also promotes IR-induced DNA damage and p53 activation, which leads to the p21-mediated G1 arrest. The IR-induced cell cycle arrest at G2/M is then shifted to G1, where cells become apoptotic in time with the p53-dependent pathways.

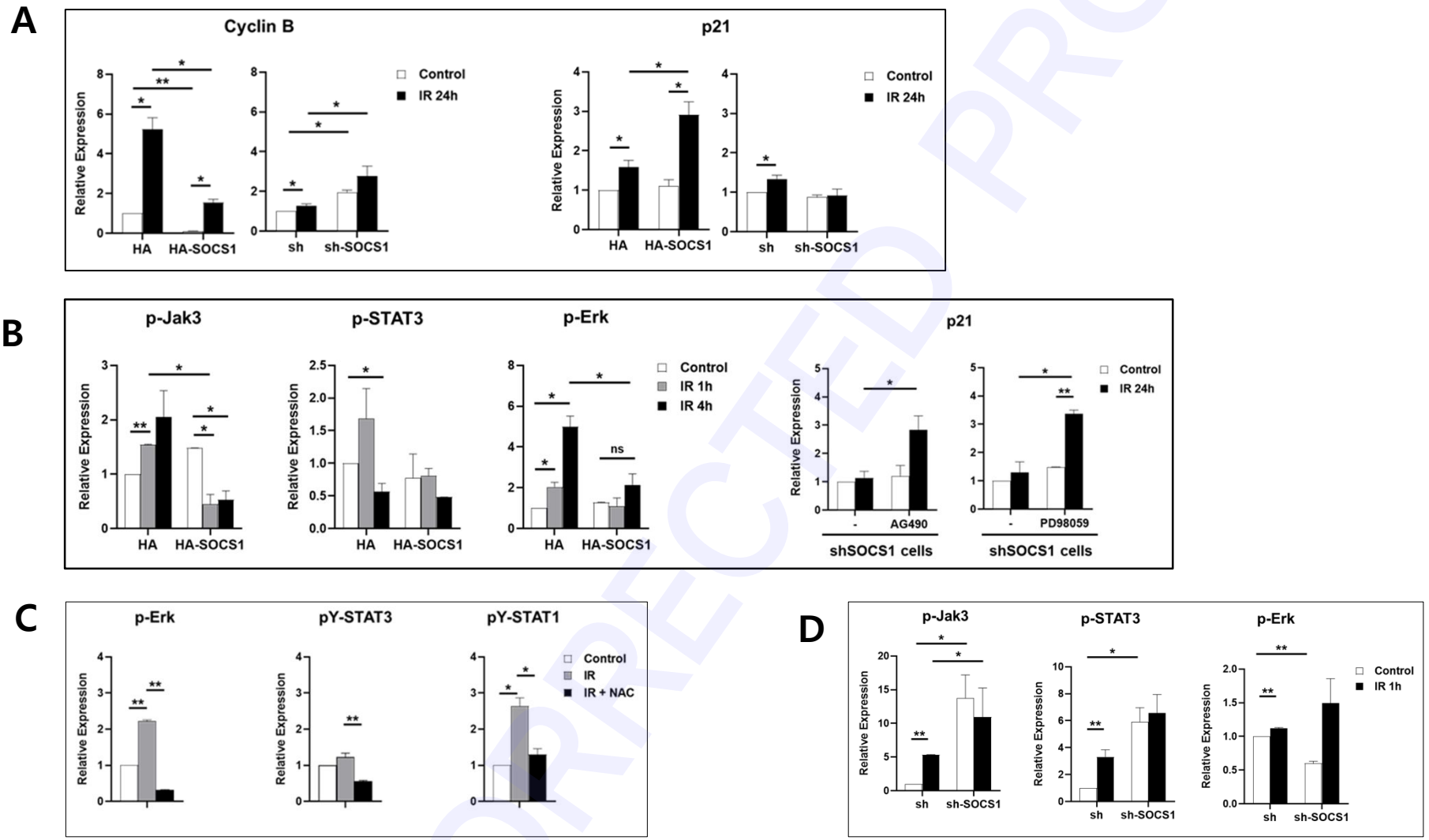

Fig S6 : Densitometric analysis of protein levels in immunoblots for Fig 2 (A), Fig 3 (B), Fig 4 (C) , and FigS4 (D). Relative expression is shown as the expression ratio determined from the band intensity of the respective protein over that of beta-actin for the untreated control taken as 1. Results show data (mean + SE) obtained from multiple blots. Statistical significance was determined by a Student's t-test. A value of * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ was considered statistically significant.