BMB Reports: 21-191

< Supplementary Materials >

SOCS1 counteracts ROS-mediated survival signals and promotes apoptosis by modulating cell cycle 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°C.

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.

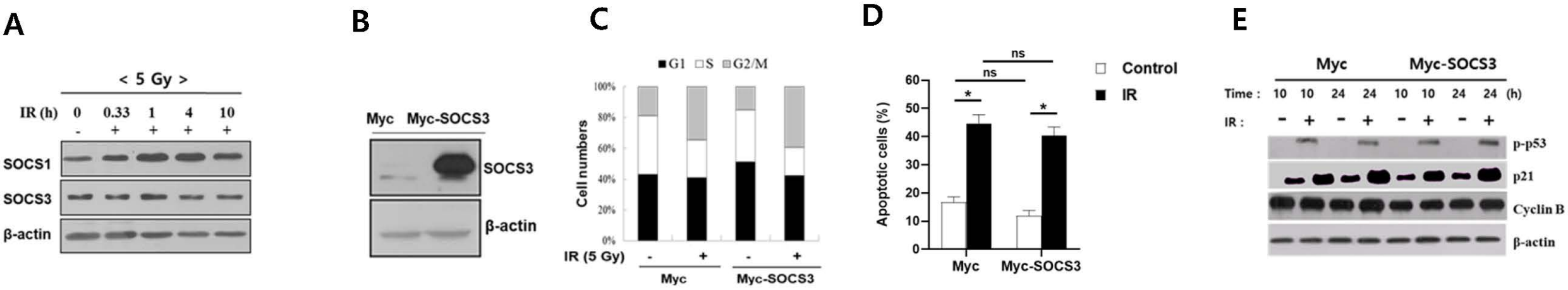

Fig S1. SOCS1 but not SOCS3 is induced in response to the IR (A) and SOCS3 over-expression does not affect the IR-induced apoptosis and cell cycle changes. HCT116/p53 wt cells were treated with γ-IR at 5 Gy and the expression levels of SOCS1 and SOCS3 were analyzed by Western blotting (A). SOCS3 over-expressing 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 in response to γ-IR were also analyzed in RKO/p53 wt CRC cells (E).

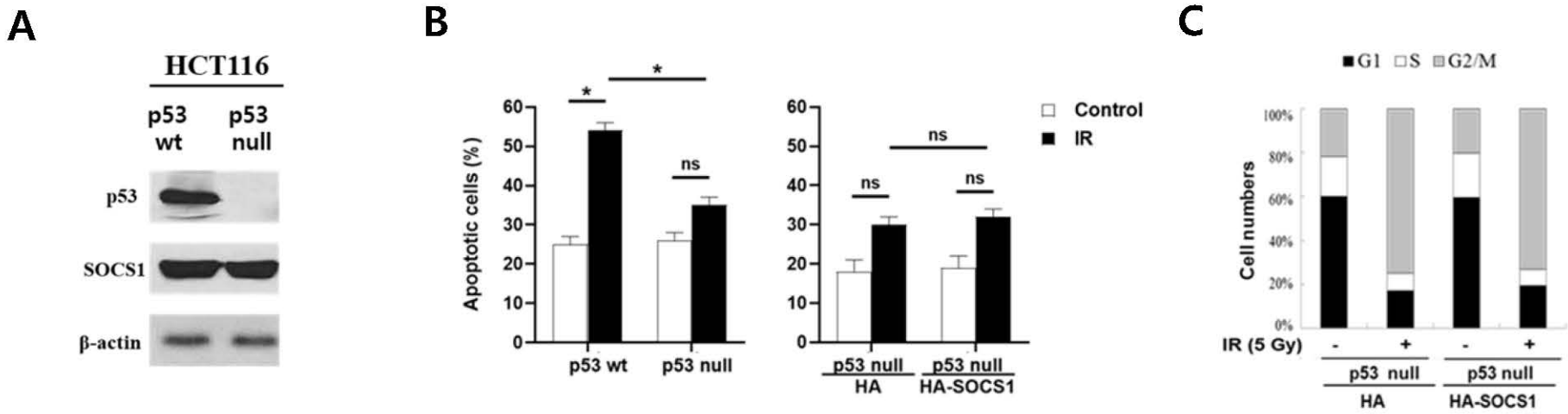

Fig S2. Apoptosis-promoting and cell cycle-modulating effects of SOCS1 is p53-dependent in HCT 116 CRC cells HCT116 /p53 wt and HCT116 /p53 null cells (A) were treated with γ-IR at 5 Gy and analyzed for apoptotic response along with HCT116 /p53 null cells stably transfected with HA or HA-SOCS1 at 48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

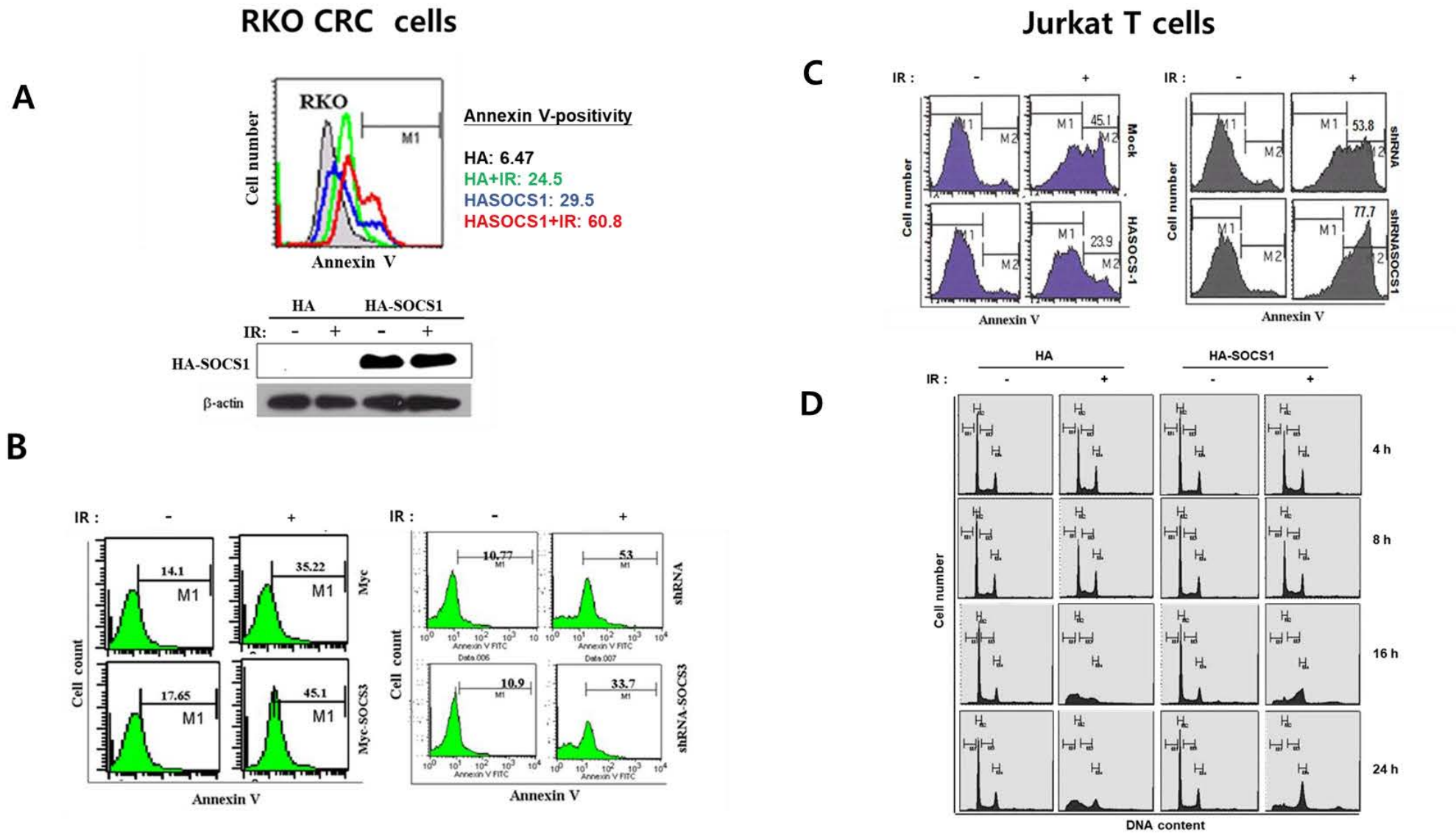

Fig S3. SOCS1 promotes radiation-induced apoptosis in RKO /p53 wt CRC cells, but exhibits opposite effects in Jurkat T/p53 mt cells with increased G2/M arrest. RKO colorectal cancer cells transfected with HA and HA-SOCS1 (A) or SOCS3 vs shSOCS3 (B) received γ-IR at 50 Gy to induce apoptosis which was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

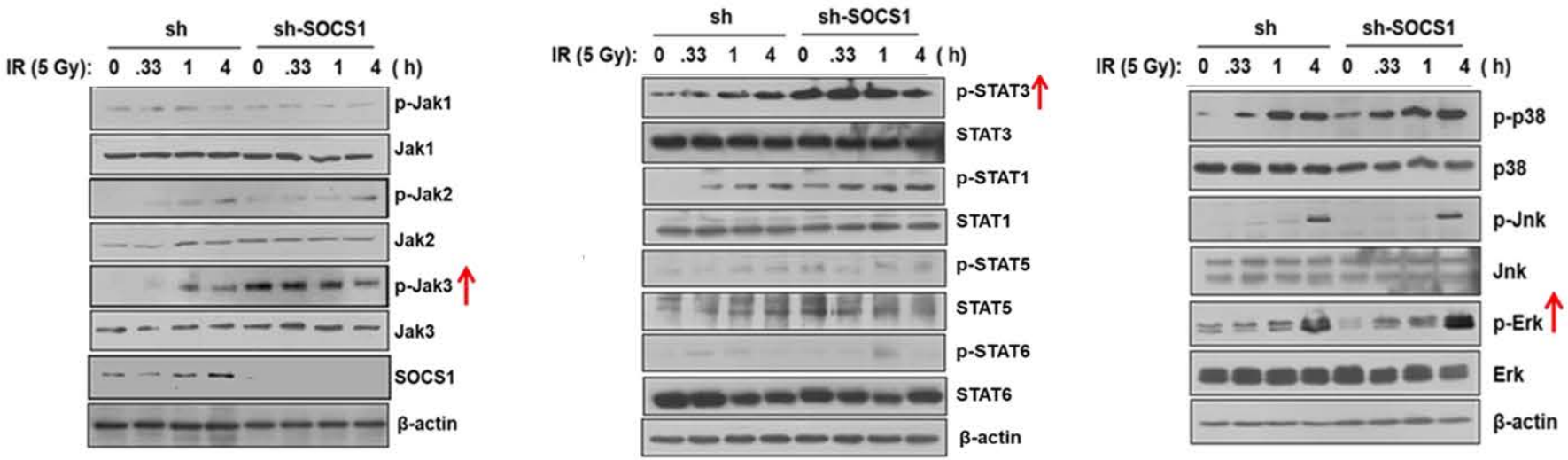

Fig S4. Analysis of Jak/STAT and MAPK activation profile induced in response to IR in sh vs shSOCS1 cells. HCT116/p53 wt cells stably transfected with sh and shSOCS1 were analyzed for Jak/STAT and MAPK activation kinetics induced by γ-IR by immunoblotting.

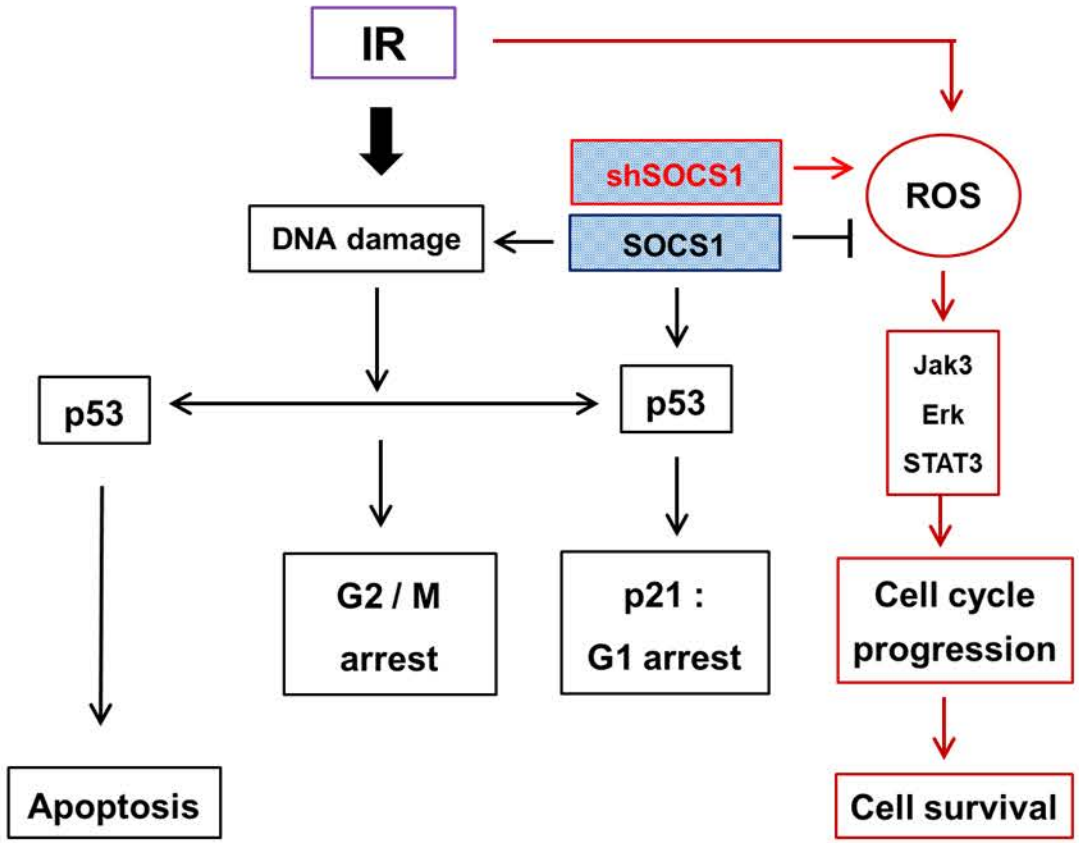

Fig S5. Schematic diagram for the regulation of the IR-induced response by SOCS1 to increase radiosensitivity of colorectal cancer cells by suppressing ROS-mediated survival pathway (red line) and promoting cell death pathway with cell cycle modulation (black line).
In addition to induce direct DNA damage, 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.

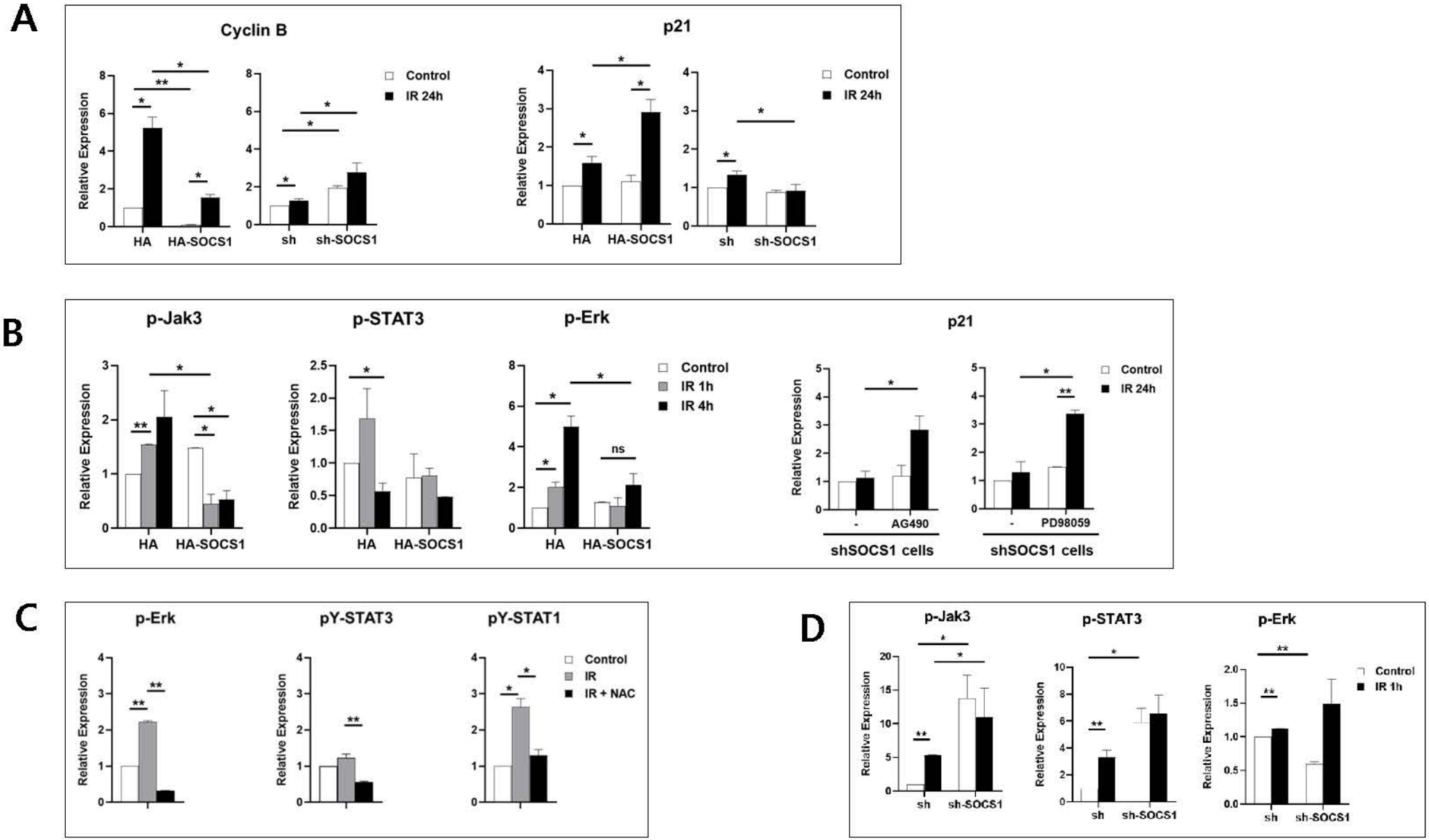

Fig S6: Densitometric analysis of protein levels in immunoblots for Fig 2 (A), Fig 3 (B), Fig 4 (C) , and FigS4 (D). Relative expression is shown as the expression ratio determined from the band intensity of the respective protein over that of beta-actin for the untreated control taken as 1. Results 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.