Supplementary Material

SOCS1 Represses Fractionated Ionizing Radiation-Induced EMT Signaling Pathways through the Counter-Regulation of ROS-Scavenging and ROS-Generating Systems

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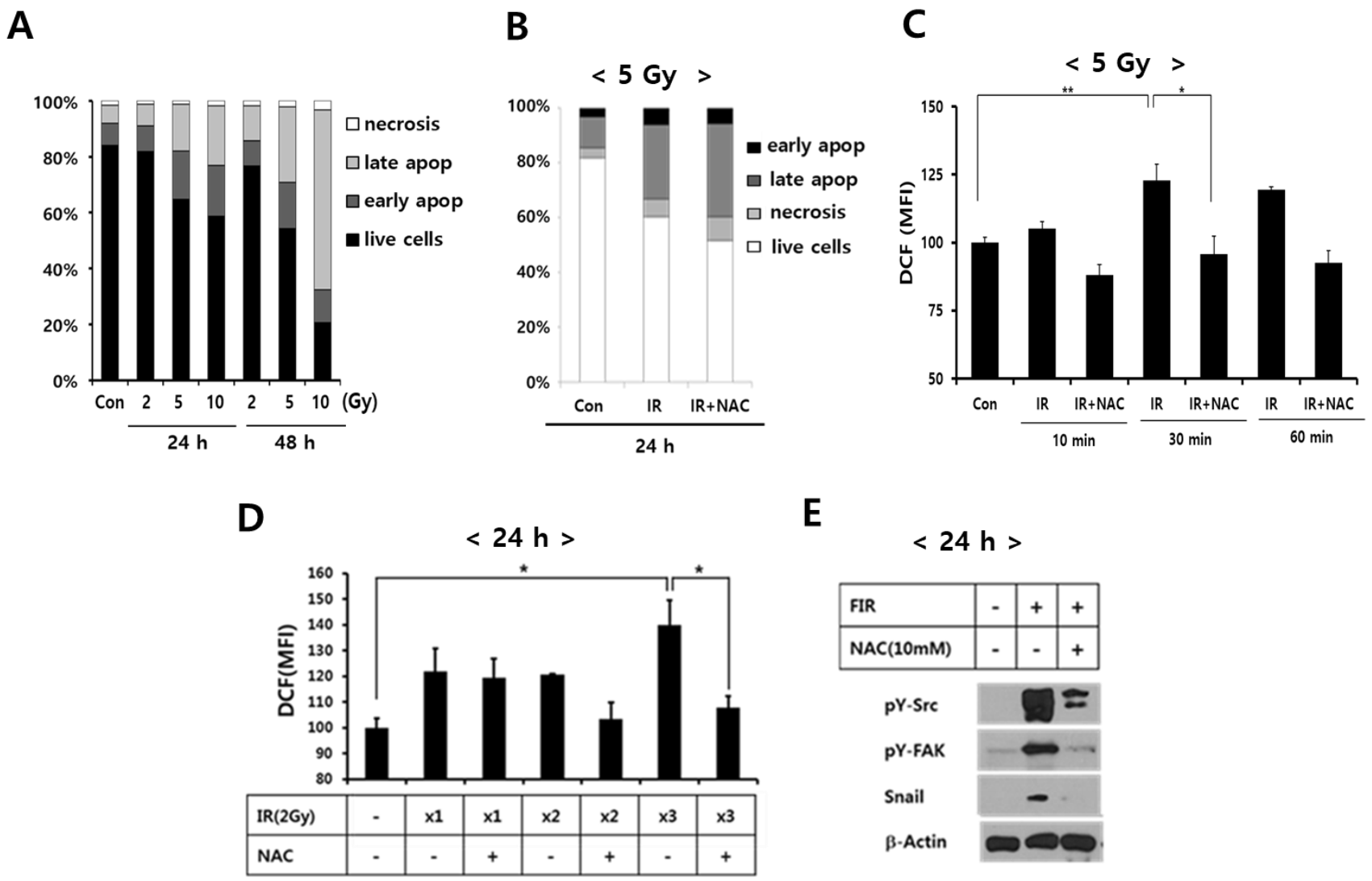

Fig S1. ROS generation by low dose irradiation correlates with resistance to the IR-induced apoptosis and EMT of HCT116 p53 $^{+/+}$ colorectal cancer cells. (A) Cells were irradiated with indicated doses of 2 to 10 Gy, and the resulting cell death was determined at 24 h and 48 h by Annexin V and PI staining. **(B and C)** The effects of NAC on the 5 Gy IR-induced ROS generation and cell death by 24 h were analyzed by FACS to determine the role of ROS on cell death. **(D)** Cells were irradiated daily at 2 Gy for 1, 2, and 3 days. The ROS levels were analyzed at 24 h after the last irradiation. **(E)** The effect of FIR (2 Gy x 3) on the induction of EMT marker expression was seen by 24 h.

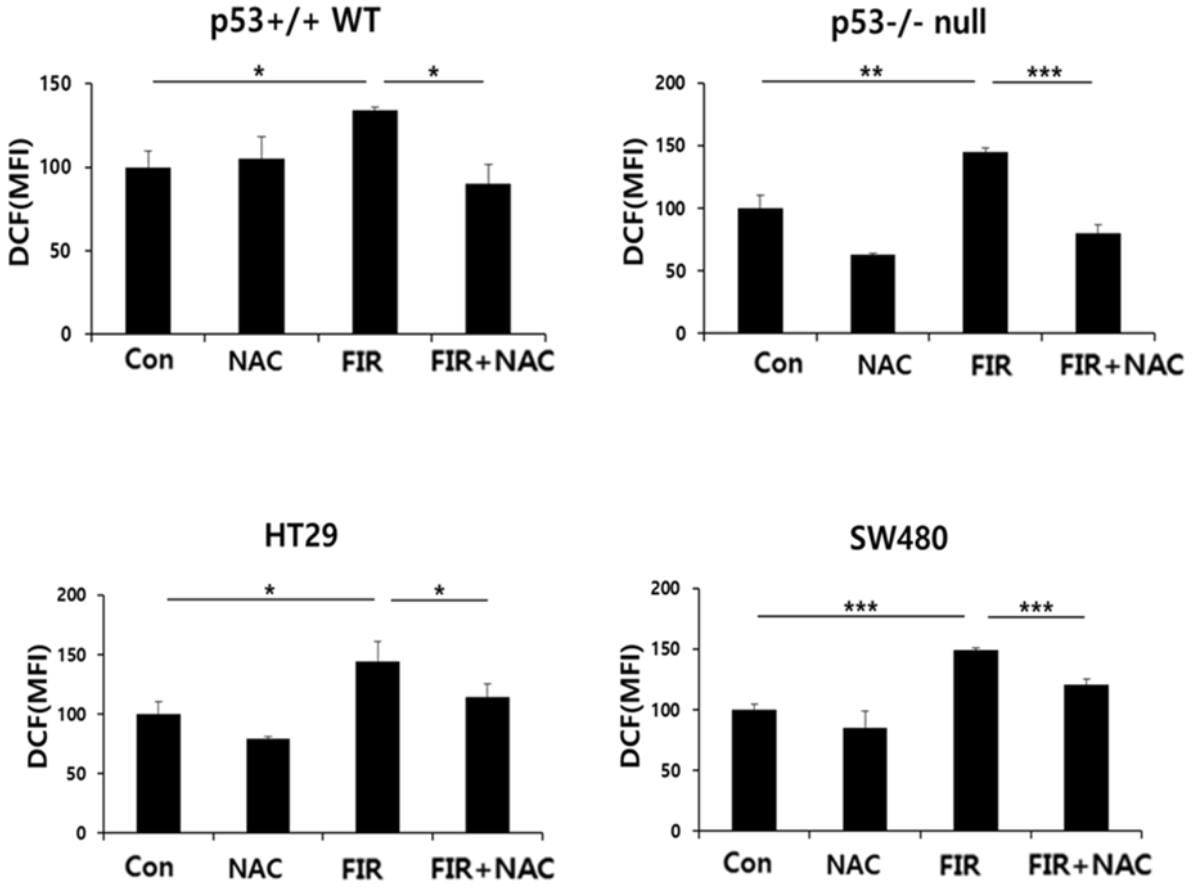

Fig S2. ROS generation by FIR is observed in various colorectal cancer cells.
Colorectal cancer cell lines HCT116 p53 (wt) $^{+/+}$, HCT116 p53 $^{-/-}$ (null) , HT29, and SW480 were cultured and subjected to FIR (2 Gy x 3), in the presence or absence of NAC treatment. Intracellular ROS levels were determined at 30 min post irradiation as described in Fig 1.

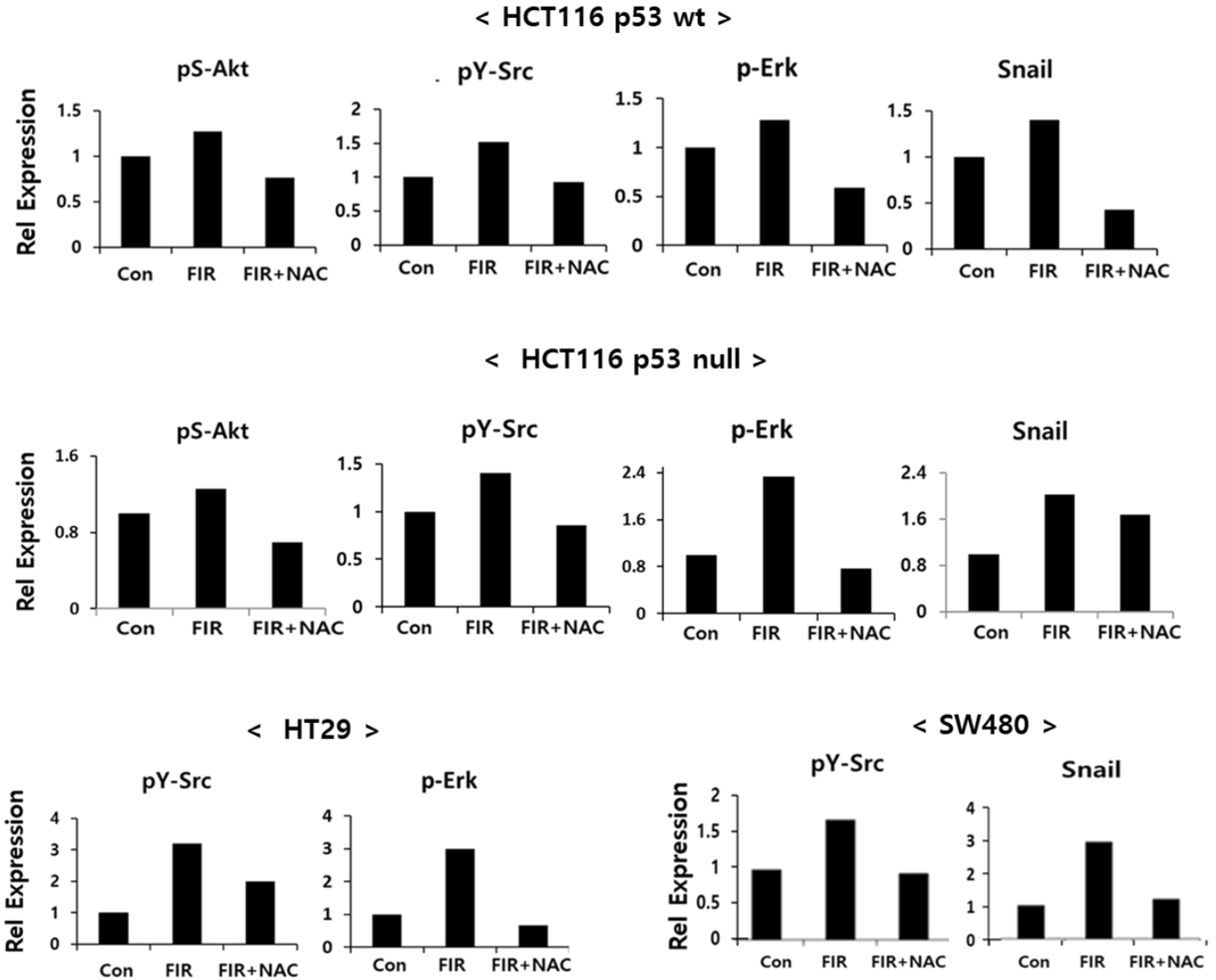

Fig S3. The FIR-induced EMT signaling is mediated by ROS in colorectal cancer cells.
HCT116 p53 $^{+/+}$(wt), HCT116 p53 $^{-/-}$ (null), HT29, and SW480 were cultured and subjected to FIR (2 Gy x 3), in the presence or absence of NAC treatment. The expression levels of EMT markers and the associated signaling molecules were determined by immunoblotting followed by densitometry analysis as described in Fig 1D.

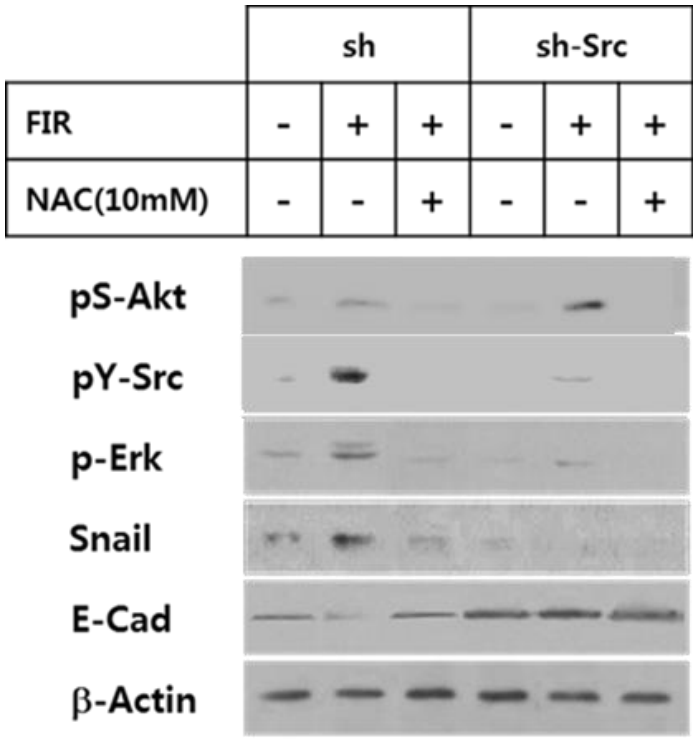

Fig S4. FIR-induced EMT signal leading to Snail/ E-cad regulation involves Akt→ Src→ Erk pathways: Role of Src. HCT116 $p53^{+/+}$ cells were transfected with shRNA against Src and subjected to FIR with or without NAC pretreatment. Western blots were performed to analyze changes in EMT markers and associated signaling molecules.

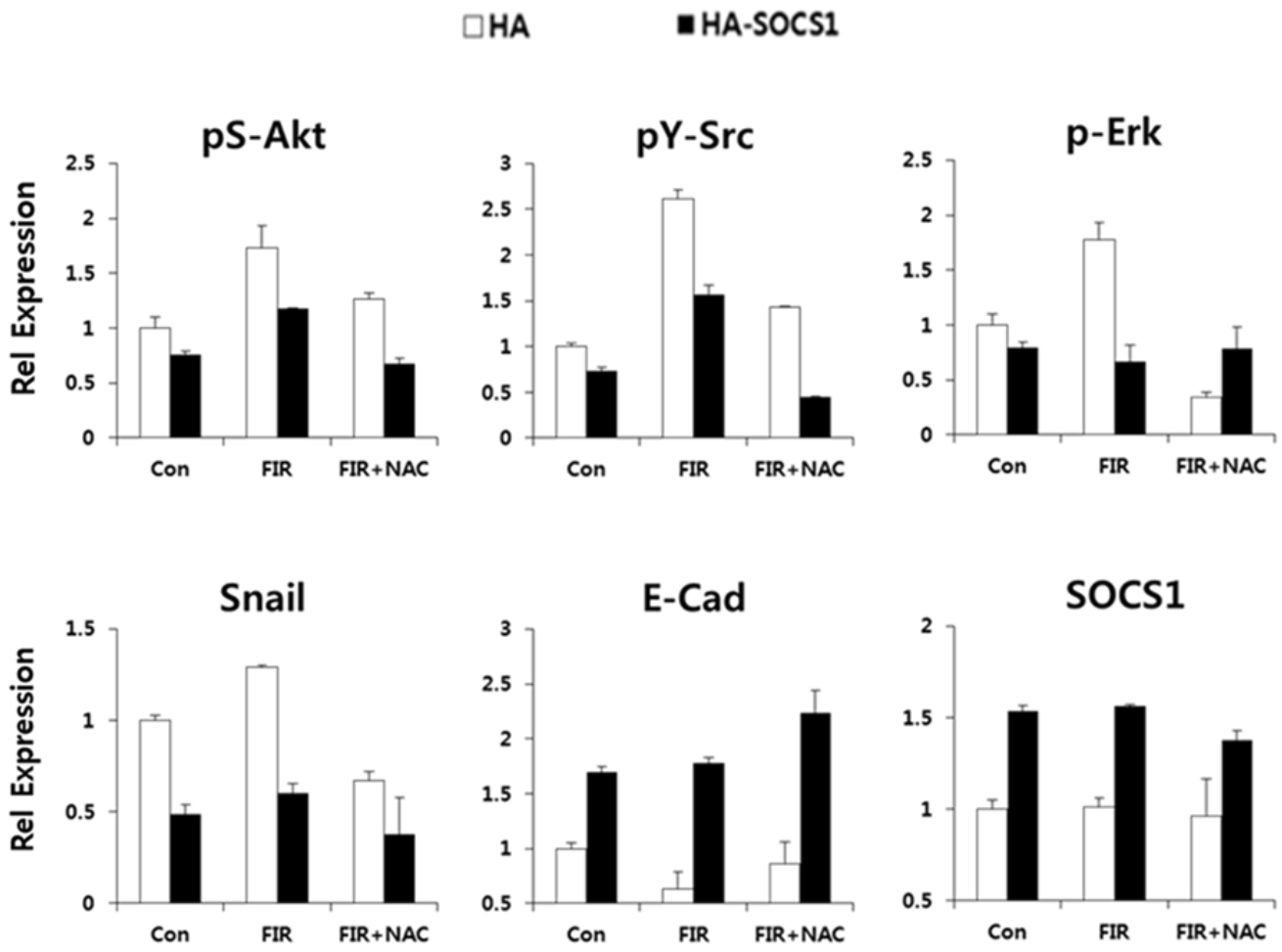

Fig S5. Inhibitory effects of SOCS1 on the FIR-induced EMT response and the associated signaling pathways. The mock (HA) vs HA-SOCS1 cells treated as in Fig 3B were analyzed for the expression levels of EMT markers and signaling molecules by Western blot. Densitometry data were obtained from multiple blots. The each expression ratio for the untreated control was taken as 1. Results show data (mean + SE) obtained from three experiments.

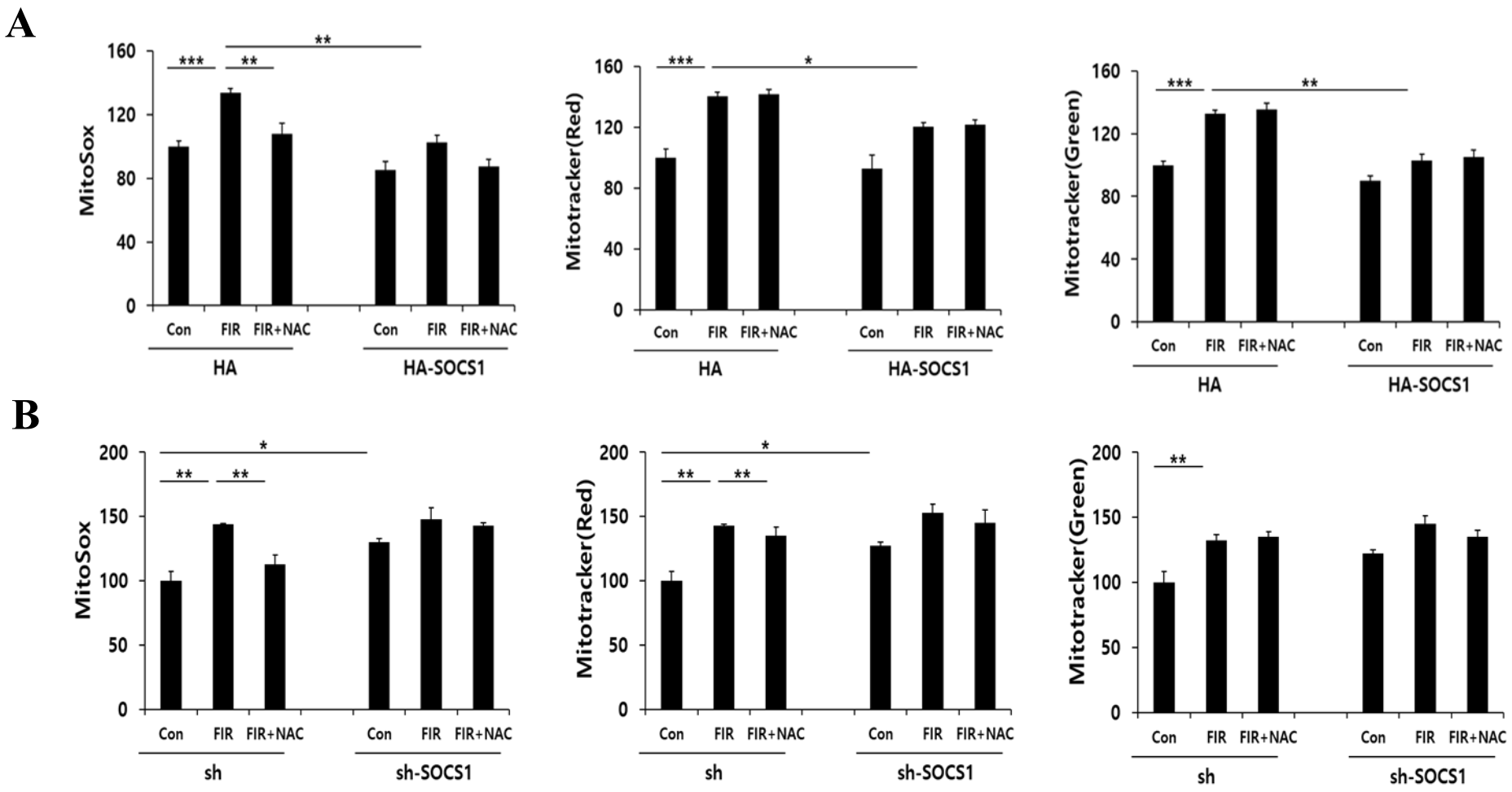

Fig S6. Effects of SOCS1 over-expression and knock-down on the mitochondrial function and ROS generation upon FIR in colorectal cancer cells.

To examine the effect of SOCS1 over-expression or knock-down on the mitochondrial ROS generation upon FIR, HA vs HA-SOCS1(A) as well as sh vs shSOCS1 (B) cells constructed from HCT116 p53 $^{+/+}$ (wt) were subject to Mitosox staining. In addition, relative levels of total mitochondria as well as mitochondria with functional ETC were determined by staining with Mitotracker green and Mitotracker red dyes, respectively. Data were obtained from two independent experiments (mean + SE) performed in triplicate wells. (*, $p \leq 0.05$; $p \leq 0.01$; ***, $p \leq 0.005$)

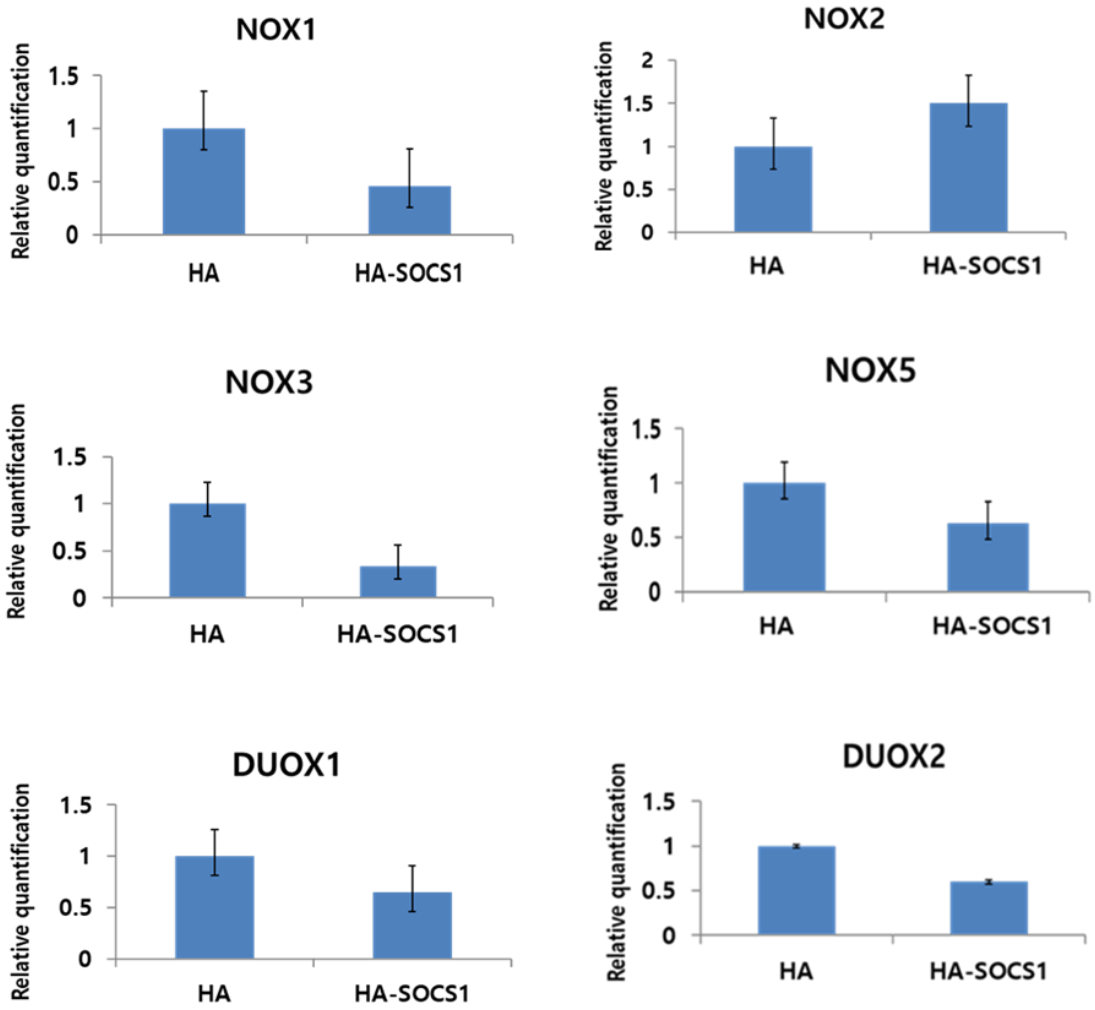

Fig S7. Analysis of Nox isozyme expression levels in SOCS1-transfected vs mock cells.
Total RNA was isolated with Trizol reagent (Invitrogen, Camarillo, CA, USA) and then reverse-transcribed, after which real-time PCR amplification with POWER SYBR® Green (Applied Biosystems, Warrington, UK) was performed using a Mastercycler realplex thermal cycler (Eppendorf AG, Hamburg, Germany). The results were normalized against GAPDH by comparing the fold change in the mRNA expression of Nox isozymes to the expression of GAPDH.