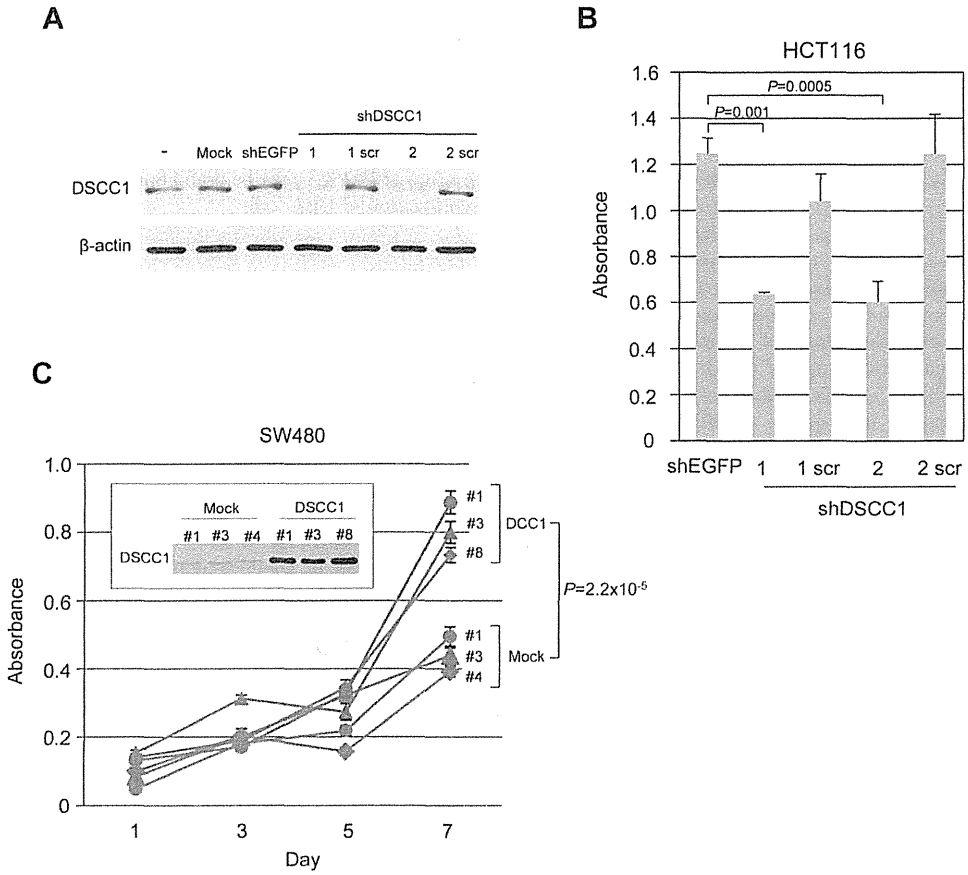

Figure 4. Involvement of DSCC1 in CRC cell proliferation. (A) HCT116 cells were transfected with control (Mock and EGFP) and DSCC1 shRNAs for 48 h using Nucleofector kit, and western blot analysis was performed. Expression of β-actin served as a control. (B) Viability of cells transfected with shRNAs was measured by WST-8 assay. The data represents mean ± SD from three independent transfections. P values were calculated with the Dunnett's test for multiple comparisons to shEGFP-transfected cells. (C) Overexpression of DSCC1 in SW480 cells was confirmed by western blotting using anti-DSCC1 antibody. Equivalent number of three mock and three DSCC1 cells was plated in 96-well plates, and cell proliferation assays were performed at the indicated time points. The data represents mean ± SD from five experiments. A significant difference between mock and DSCC1 cells was determined by two-way repeated measures ANOVA.
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proteins, and histone-modification enzymes. Therefore, other factor(s) might affect the elevated promoter activity by E2F4. Although the direct association of E2Fs and their cofactors with the three binding sites needs future detailed analysis, the region containing the three should play a vital role in the elevated expression of DSCC1.

We here showed for the first time that DSCC1 plays an important role in survival of human cancer cells, since enhanced expression of DSCC1 induced survival of cancer cells in response to γ-irradiation, topoisomerase I inhibitor, and DNA-intercalator. The data are consistent with the finding that Dscc1 mutants exhibit sensitivity to γ-irradiation in *Saccharomyces cerevisiae* [27,28]. Another study showed that repair of a topoisomerase I inhibitor-induced DNA double-strand breaks, required components of chromatid cohesion including *Csm3*, *Tof1*, *Mrc1*, and *Dscc1* [29]. Alternatively, DSCC1 may enhance the recombination repair through the CTF18-RFC complex. Our study additionally showed that this resistance seems to be independent of p53 because the induction of apoptosis was also potentiated in HCT116 p53−/− cells (Figure S3F). Associated with CTF8, DSCC1 forms an alternate RFC with CTF18, and further stabilizes 7-subunit complex with RFC2, RFC3, RFC4, and RFC5. Depletion of DSCC1 reduces expression of CTF18, induces decreased replication fork, increases collapse, and suppresses recovery of forks to replication inhibitors, suggesting that DSCC1 is important for DNA replication and recovery from genotoxic insults [30].

Global gene-gene interaction studies have helped gain insights into the complex genetic networks in the yeast. These studies disclosed synthetic lethal combinations of genetic dysfunction, where two genetic variations that have individually no effect on cell viability cause cell death if combined. The concept of synthetic lethality is of great importance in creating therapeutic approaches to selectively kill cancer cells, because genetic and/or epigenetic alterations are expected in cancer cells but not in noncancerous cells. For example, PARP inhibitors have been shown to induce synthetic lethality to cancer cells with BRCA1 or BRCA2 mutations [31,32]. Of note, McLellan and colleagues validated genetic interactions of synthetic lethality in the yeast between *ctf8*, *ctf18*, *dscc1*, *ctf4*, and *rad27* with genes required for the maintenance of chromosomal stability [6]. They additionally showed that these genetic interactions are conserved in *Caenorhabditis elegans*, suggesting the potential utility of these genes for the

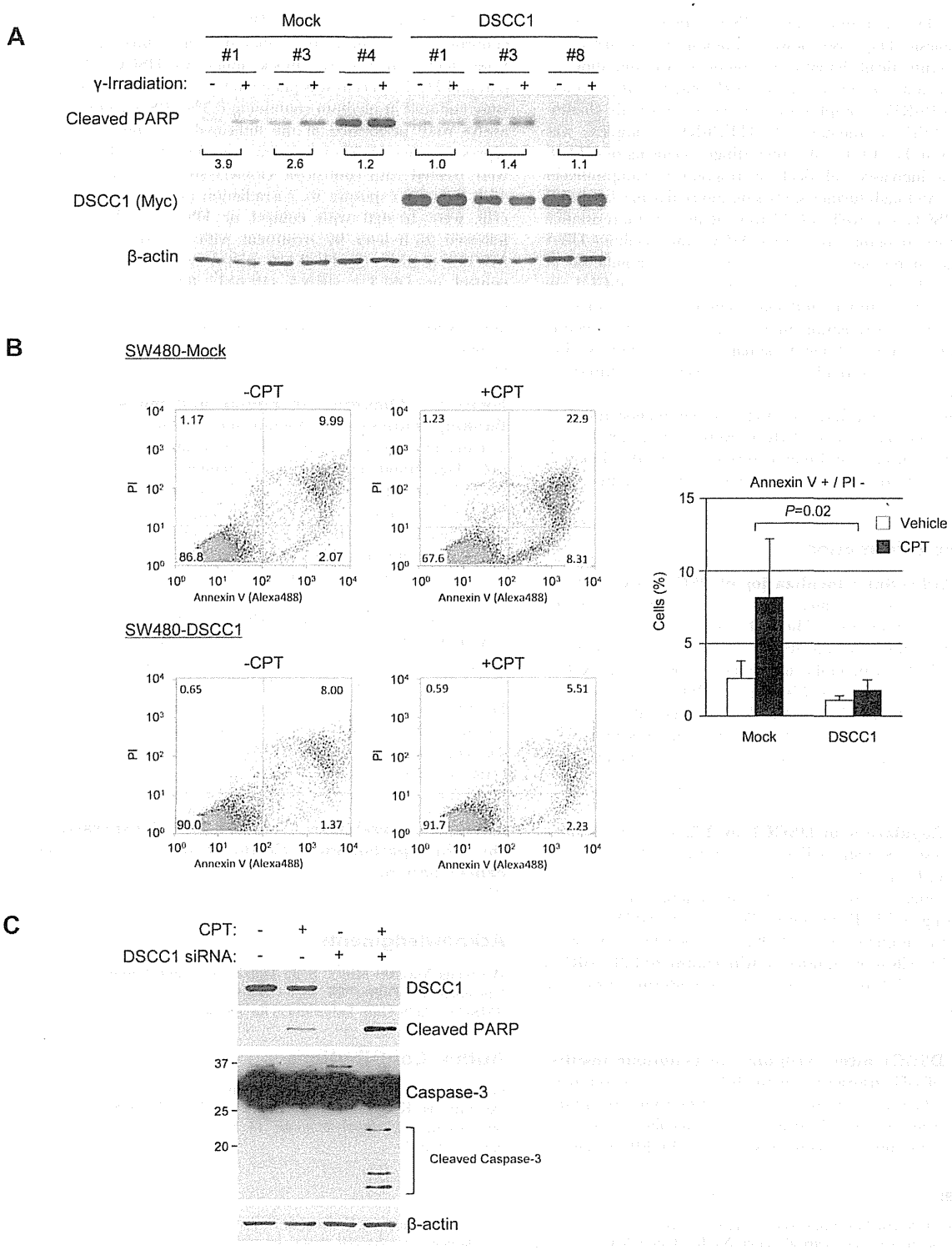

Figure 5. DSCC1 alters sensitivity to apoptotic stimuli. (A) SW480 cells stably expressing DSCC1 or mock (empty vector) were exposed to γ-irradiation (5 Gy). The cells were harvested 24 h after exposure, and the lysates were subjected to western blot analysis. (B) SW480 cells stably expressing DSCC1 or mock were treated with camptothecin (CPT, 30 μM). The cells were harvested 24 h after treatment, and the cell suspensions were subjected to annexin V staining. The data represents mean ± SD from three different clones. Increased annexin V-positive cell population by treatment with CPT was compared between control (Mock) and DSCC1-expressing cells. A significant difference was determined by t-test. (C) HCT116 cells were transfected with control or DSCC1 siRNA, and treated with CPT (30 μM) at 48 h. The cells were harvested 24 h after the CPT-treatment, and the lysates were subjected to western blot analysis.
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treatment of colorectal tumors where CIN is frequently involved in the carcinogenesis. They also showed mutations in *ctf4*, *ctf8*, *ctf18*, and *dscc1* are synthetically lethal when combined with mutations in CIN genes including *mre11*, *smc1*, *smc3*, *scc2*, and *pds1* [6]. To test whether CTF18-RFC complex may be associated with chemosensitivity, CTF18, a member of CTF18-RFC complex, was knocked down in HCT116 cells. Interestingly, silencing of CTF18 resulted in the increased cell death in response to camptothecin (Figure S4C). Although further studies on molecular mechanism(s) underlying DSCC1- as well as CTF18-mediated chemoresistance are needed, these data may imply that DSCC1 may facilitate DNA repair through homologous recombination by the regulation of this complex. If this is the case, inhibition of DSCC1 in combination with treatment inducing genotoxic insults such as camptothecin and γ-irradiation may be an effective therapeutic option. Comprehension of DNA damage, repair activities, and anti-apoptotic abilities should be needed to clarify the threshold for apoptosis in each cell.

In summary, our data may give a clue to the understanding of new molecular mechanisms underlying resistance of cancer cells against genotoxic insults, and may contribute to the development of new strategies to overcome the chemoresistance to anti-cancer drugs.

Supporting Information

Figure S1 Subcellular localization of DSCC1. (A) HCT116 and SW480 cells were treated with MG132 (10 μM, 6 h) or cycloheximide (100 μg/ml). The cells were harvested at the indicated time points, and the lysates were subjected to western blot analysis. (B) High magnification images of Figure 1D (x180). (C) HCT116 cells expressing Myc-tagged DSCC1 were probed with anti-Myc antibody followed by FITC-conjugated anti-mouse IgG secondary antibody (green). Nuclei were counter-stained with DAPI (blue). (D) The cytoplasmic and nuclear proteins were analyzed by western blotting.
(TIF)

Figure S2 Regulation of DSCC1 by E2Fs. (A) HEK293T cells were transfected with pcDNA3 HA-E2F1, -E2F2, -E2F3, -E2F4, and -E2F6 for 24 h, and the lysates were subjected to western blot analysis. (B) Chromatin immunoprecipitation was performed using anti-E2F1 antibody. The precipitated DNAs were subjected to the amplification of *CDC2* promoter by quantitative PCR. (C) HeLa cells were transfected with control or E2F1 siRNA (25 nM) for 48 h. Western blot analysis was performed using the indicated antibodies.
(TIF)

Figure S3 DSCC1 alters response to genotoxic insults. (A) Viability of cells transfected with shRNAs was measured by WST-8 assay. The data represents mean ± SD from three to five independent transfections. P values were calculated with the Dunnett's test for multiple comparisons to shEGFP-transfected cells. (B) Overexpression of DSCC1 in HCT116 cells was confirmed by western blot analysis with anti-Flag antibody. Equivalent number of two mock clones, two DSCC1 clones, and parental HCT116 cells was plated in 96-well plates, and these cells were cultured in medium containing 0.5% FBS. Cell proliferation assays were performed at the indicated time points. The data represents mean ± SD from eight experiments. (C) HCT116 cells were treated with control or DSCC1 siRNA (10 nM), followed 48 h later by exposure to γ-irradiation (5 Gy). (D, E) HCT116 cells were treated with control or DSCC1 siRNA (10 nM), followed 48 h later by treatment with doxorubicin (5 μM) or MG132 (2 μM). (F) HCT116 p53-/- cells were treated with control or DSCC1 siRNA (10 nM), followed 48 h later by exposure to γ-irradiation (5 Gy). The cells were harvested 24 h after exposure, and the lysates were subjected to western blot analysis.
(TIF)

Figure S4 Alignment of human and mouse *DSCC1* 5′-flanking sequence. (A) Alignment of human and mouse *DSCC1* 5′-flanking sequence by the DBTSS database (http://dbtss.hgc.jp/). Top strand represents the 5′-flanking sequences of human *DSCC1*, and the bottom strand represents the 5′-flanking sequences of mouse *Dscc1*. E2F binding motifs are underlined. (B) pDSCC1-133/+109 or the shorter promoter constructs was transfected with pRL-TK into SW480 cells. The promoter activity was measured by luciferase activity. Each value represents mean ± SD from three independent transfections. (C) The effect of CTF18 siRNA (S: 5′-CCAACUGCCUGGUCAUCG-3′, AS: 5′-UCGAUGACCAGGCAGUUG-3′) was evaluated by quantitative PCR (CTF18 primers, forward: 5′-CTTCTCGGTGTGGCAGGA-3′, reverse: 5′-CAGCAGGAGTGTGTCAGCAG-3′). HCT116 cells were treated with control or CTF18 siRNA (10 nM), followed 48 h later by treatment with CPT (30 μM). The cells were harvested 24 h after treatment, and the lysates were subjected to western blot analysis.
(TIF)

Table S1 Correlations between DSCC1 expression and the clinicopathological characteristics of the 40 colon cancer patients.
(XLS)

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Author Contributions

Conceived and designed the experiments: KY YF. Performed the experiments: KY NT. Analyzed the data: RY AN SI SM. Contributed reagents/materials/analysis tools: TI TF MS GT KH YN. Wrote the paper: KY YF.

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