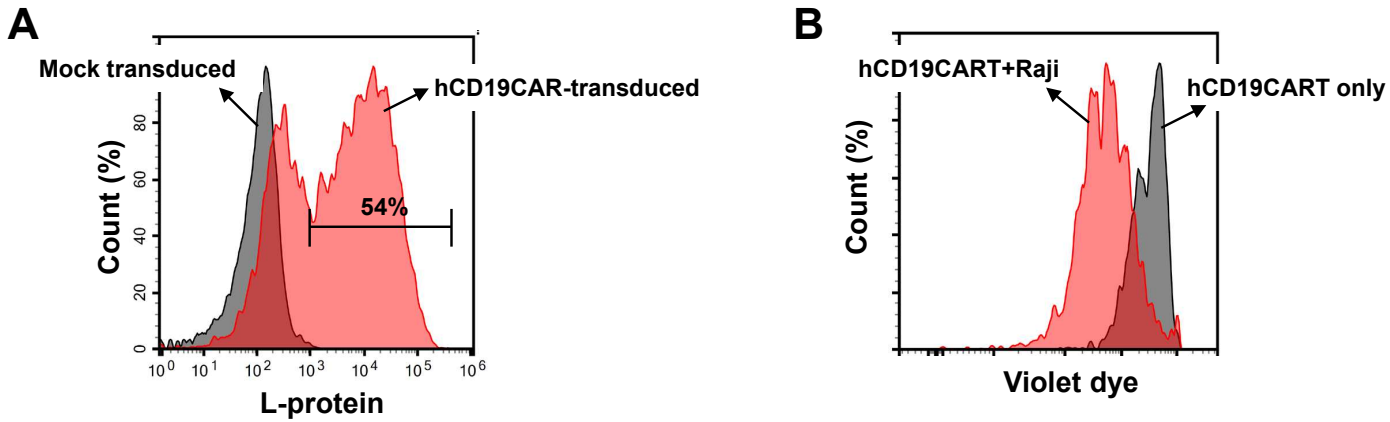
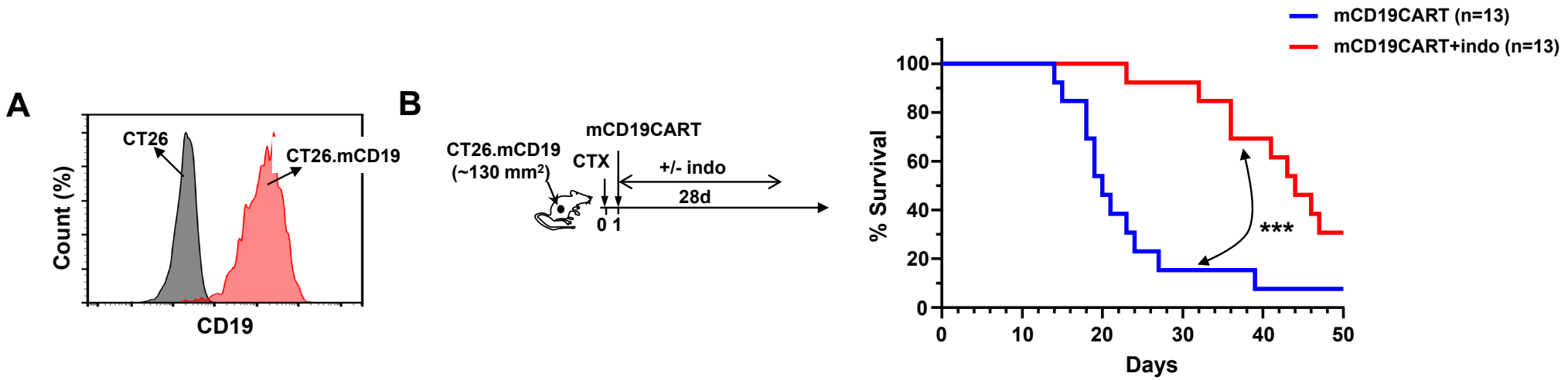
Supplemental Materials

Supplemental Figure 1 Preparation of human CD19CAR T cells. (A) Human T cells isolated from the PBMCs of a healthy donor were transduced to express human CD19-targeting CAR (hCD19CAR) using retroviral vector SFG-humCD1928z. The transduction efficacy was evaluated by Protein L staining. Mock-transduced T cells were used as a control. (B) hCD19CAR T cells are reactive to Raji tumor cells in vitro. Violet dye-labeled hCD19CAR T cells were either cultured alone or with irradiated Raji tumor cells. T cell proliferation as a result of antigen recognition was reflected by reduction of violet dye intensity.



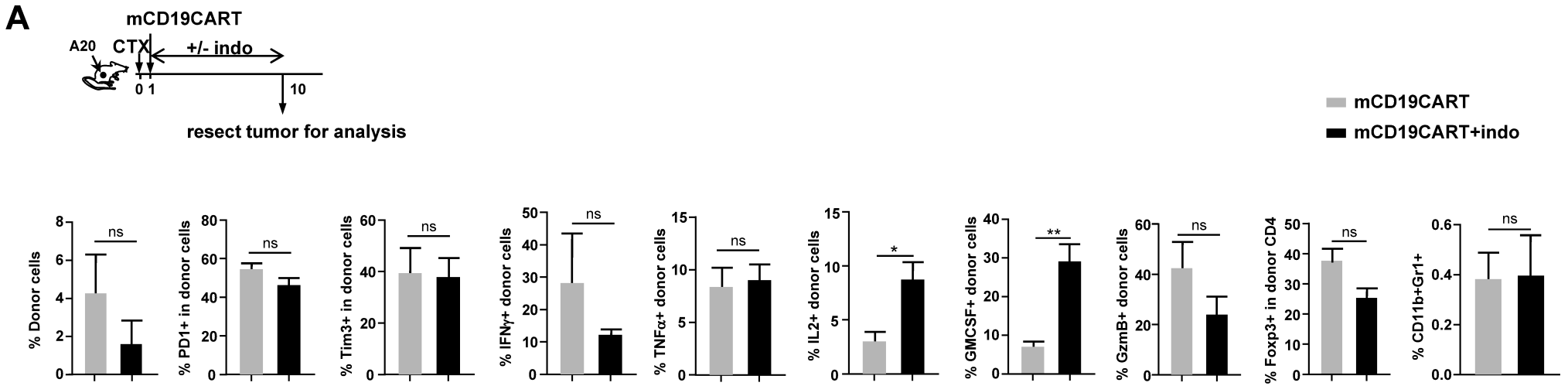
Supplemental Figure 1.

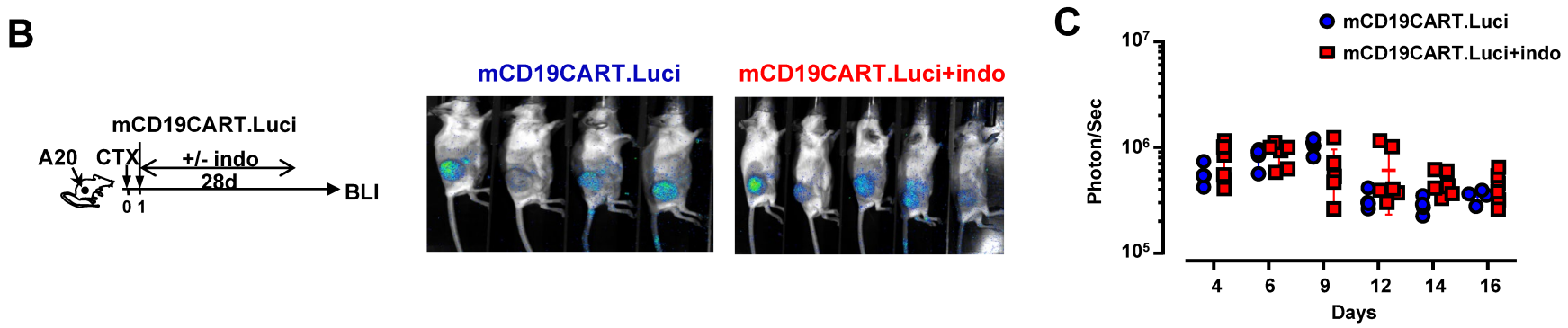
Supplemental Figure 2 Indomethacin potentiates the efficacy of ACT in a solid tumor model. (A) Confirmation of CD19 expression in CT26.mCD19 cells. Murine colorectal cancer CT26 cells were virally transduced to express murine CD19. $CD19^+$ cells were expanded after FACS sorting. The histogram shows the level of CD19 expression in CT26.mCD19 cells compared to that in parental CT26 cells. (B) Indo administration improves the therapeutic outcome of mCD19CART in mice with advanced CT26.mCD19 tumors. Following the experimental procedures depicted in the schema, Mice with established CT26.mCD19 tumors ($130mm^2$) were conditioned with CTX followed by adoptive transfer of mCD19CAR T cells. A cohort of mice received daily indo administration for 28 days. Tumor growth was monitored by caliper measurement and mice were euthanized when tumor diameter reached 20mm. Mouse survival results are summarized in Kaplan-Meier curves. Data shown are pooled from two independent experiments. Statistics: (B) Log-rank (Mantel-Cox) test. ***$P < 0.001$.



Supplemental Figure 2.

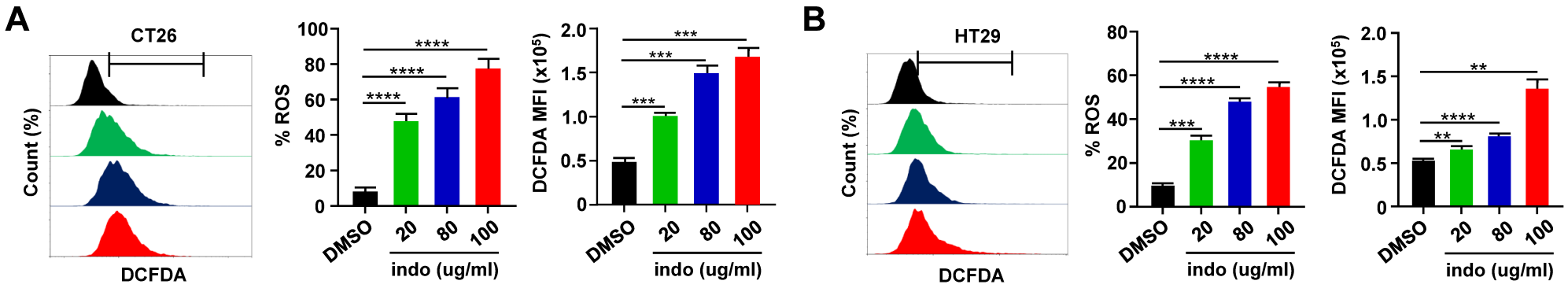
Supplemental Figure 3 The impact of indomethacin on the phenotype, function and tumor infiltration of mCD19CAR T cells. (A) Phenotypical and functional characterization of tumor-infiltrating mCD19CAR T cells. The schema depicts the experimental procedures. Mice with established A20 tumors received CTX-conditioning followed by adoptive transfer of CD45.1$^+$ mCD19CAR T cells, and a cohort of mice received additional indo administration. On day 10, tumor tissues were collected and processed into single cell suspensions for analysis. Donor T cells in tumor were identified as CD45.1$^+$ cells. Cells were subjected to FACS analysis to evaluate the frequency, activation/exhaustion markers (PD1 and Tim3), and functionality (IFNg, TNFa, IL2, GMCSF and GzmB) of the donor T cells, as well as the frequencies of Tregs (CD4$^+$Foxp3$^+$) and MDSCs (CD11b$^+$Gr1$^+$) in tumors. The cytokine profile of donor T cells were determined by ICS after stimulating T cells with PMA/ionomycin in the presence of GolgiPlug for 3.5 hours. The results are summarized in bar graphs and shown as mean ± SEM of three samples per condition. Statistics: Unpaired t-test. *P < 0.05, **P < 0.01, ns: non-significant. (B) Tumor infiltration kinetics of mCD19CAR T cells revealed by BLI analysis. Following the procedures depicted in the schema, T cells co-transduced with mCD19CAR and luciferase were transferred to CTX-conditioned A20-bearing mice followed by indo administration to a cohort of mice for 28 consecutive days. BLI was conducted periodically to visualize luciferase-expressing mCD19CAR T cells in live mice. Representative images of mice in each group are shown. Results of intratumoral donor T cell luciferase signal intensity quantified as mean ± SEM are summarized in (C). Each symbol in the plot represents one mouse.





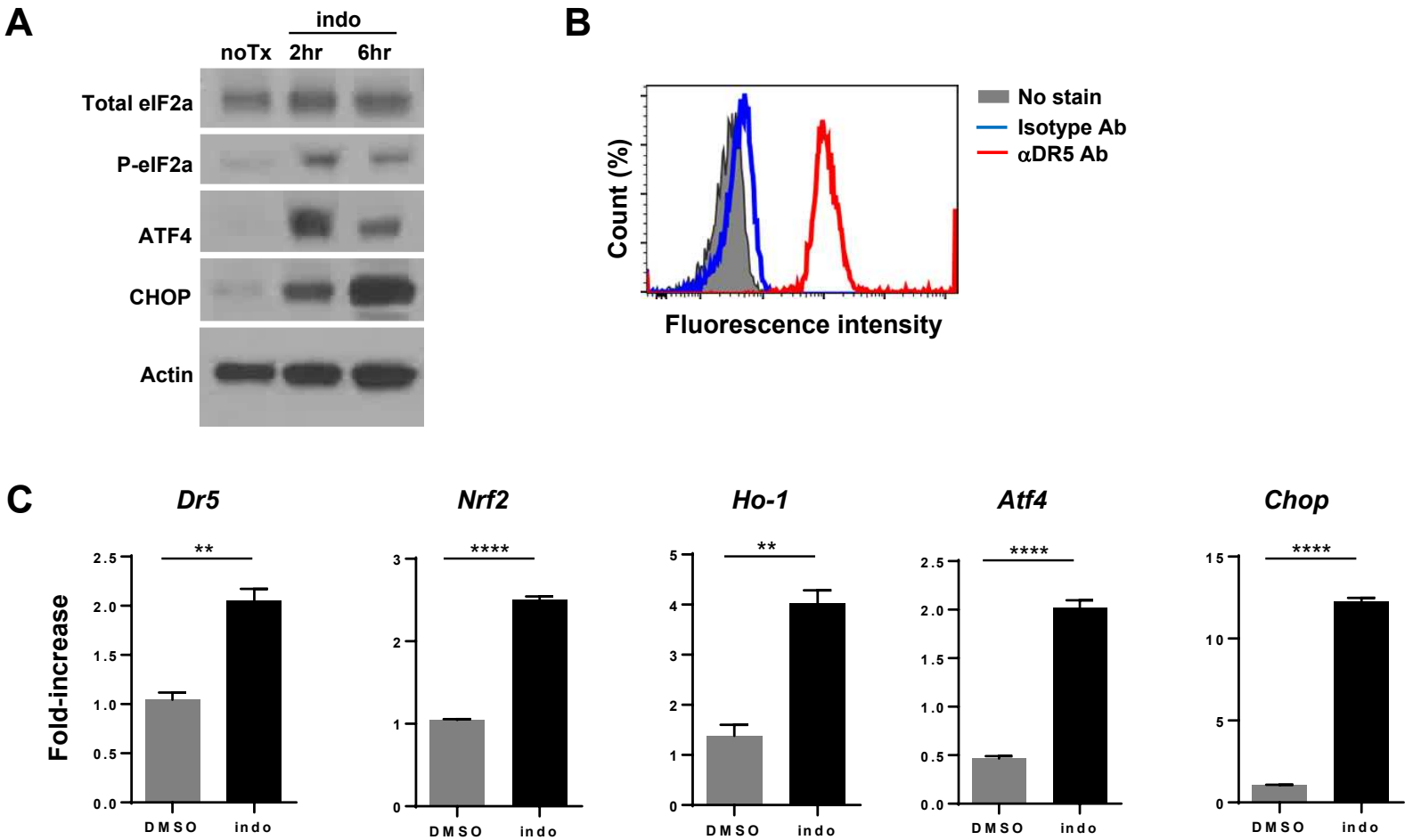
Supplemental Figure 3.

Supplemental Figure 4 Indomethacin induces oxidative stress in solid tumor cell lines. (A-B) Human colon cancer HT29 cells and murine colorectal cancer CT26 cells were cultured in a 24-well plate overnight to allow cell attachment. Cells were treated with DMSO or varying doses of indo as indicated. After overnight culture, cells were harvested and stained with CM-H2DCFDA to evaluate ROS levels. Representative histograms show overlay of CM-H2DCFDA curves. A gate is arbitrarily placed to denote % ROS^+ cells. Results of % ROS^+ cells under the indicated conditions are summarized in bar graphs and shown as mean ± SEM of triplicate samples. The MFIs of CM-H2DCFDA signals are also summarized in bar graphs and shown as mean ± SEM of triplicate samples. Data shown are representative of three independent experiments with similar results. Statistics: One-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.



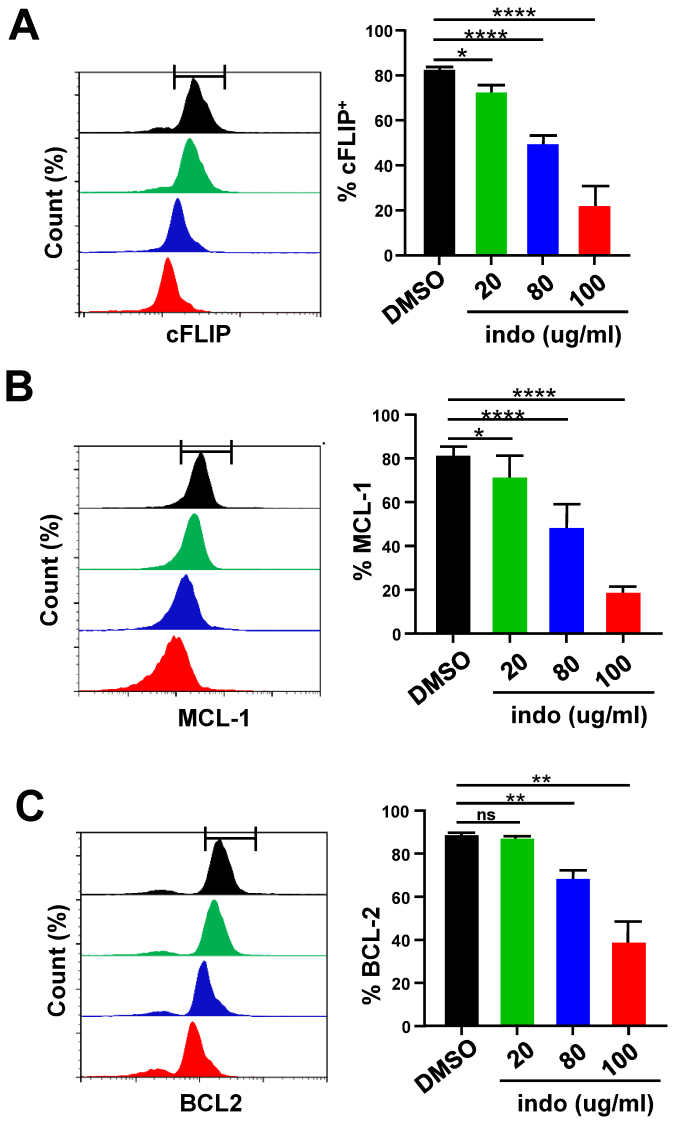
Supplemental Figure 4.

Supplemental Figure 5 Indomethacin induces oxidative/ER stress and boosts DR5 expression in A20 cells. (A) Induction of molecules associated with oxidative and ER stress in indo-treated A20 cells. A20 cells were treated with 80µg/ml indo for 2 hours or 6 hours. Cells were harvested for Western blot to detect the indicated ER stress markers. Data shown are representative of two independent experiments with similar results. (B) A20 cells constitutively express DR5. A20 cells were either unstained, or stained with anti-DR5 or an isotype antibody after incubating with 2.4G2 anti-CD16/CD32 Fc blocking antibody. Histogram overlay shows the basal level of DR5 in A20 cells. (C) Detection of increased transcripts of DR5 and stress response genes in indo-treated A20 cells. A20 cells were untreated, or treated with DMSO or indo (80µg/ml) overnight. Cells were harvested for RNA extraction and subjected to quantitative real-time PCR to evaluate the mRNA levels of the indicated genes. The target gene transcripts were normalized to β-actin. Transcripts fold-changes in treated samples relative to untreated samples are summarized in bar graphs and shown as mean ± SEM of triplicate reactions. Statistics: Unpaired t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.



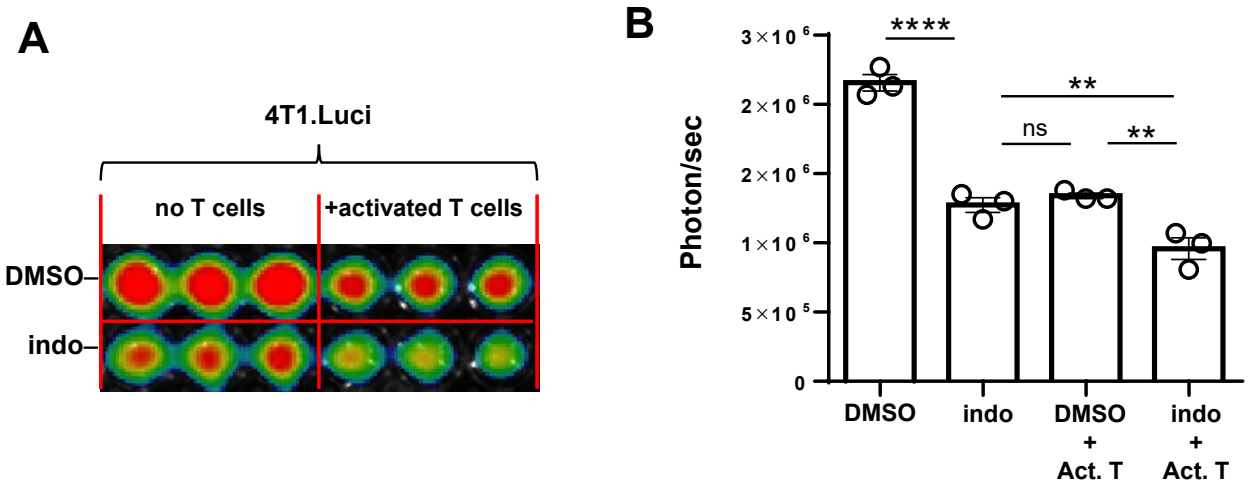
Supplemental Figure 5.

Supplemental Figure 6 Indomethacin treatment leads to downregulation of anti-apoptotic molecules in tumor cells. A20 cells were treated overnight with DMSO or varying doses of indo as indicated. Cells were harvested and stained for cFLIP (A), MCL-1 (B) and BCL-2 (C). Histograms show overlay of target molecule expression in A20 cells under the indicated conditions. A gate is arbitrarily placed to denote % of cells positive for the target molecule. Results are summarized in bar graphs and shown as mean ± SEM of triplicate samples per condition. Data shown are representative of two independent experiments with similar results. Statistics: One-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: non-significant.



Supplemental Figure 6.

Supplemental Figure 7 Indomethacin sensitizes 4T1 tumor cells to T cell cytotoxicity in vitro. (A) Luciferase-expressing 4T1 cells (4T1.Luci) were seeded to a 96-well plate at the density of 25,000 cells/well to allow cell attachment after overnight culture. Then 4T1.Luci cells were cultured alone, or co-cultured with an equal number of activated T cells, in the presence of DMSO or indo (40 ug/ml). For T cell preparation, T cells isolated from a BALB/c mouse were stimulated with CD3/CD28 Dynabeads for 72 hours to generate activated T cells. After overnight incubation of T cells with 4T1.Luci cells, BLI was performed to record the bioluminescent photon intensity in each well. Results are summarized in bar graphs and shown as mean ± SEM of triplicate samples per condition (B). Statistics: One-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01, ns: non-significant.



Supplemental Figure 7.

Supplemental Table 1: List of primers used for quantitative reverse transcriptase PCR.

Primers	**Sequence**
Dr5-F	CGGGCAGATCACTACACCC
Dr5-R	TGTTACTGGAACAAAGACAGCC
Nrf2-F	CGAGATATACGCAGGAGAGGTAAGA
Nrf2-R	GCTCGACAATGTTCTCCAGCTT
Atf4- F	ATGGCCGGCTATGGATGAT
Atf4- R	CGAAGTCAAACTCTTTCAGATCCATT
Ho-1F	CACGCCAGCCACACAGCACTA
Ho-1R	GGCTGTCGATGTTCGGGAAGG
Chop-F	CTGCCTTTCACCTTGGAGAC
Chop-R	CGTTTCCTGGGGATGAGATA
Actin	AGAGGGAAATCGTGCGTG
Actin	CAATAGTGATGATGACCTGGCCGT