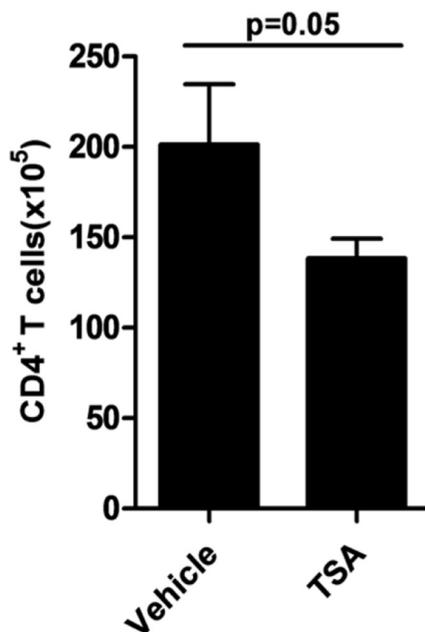
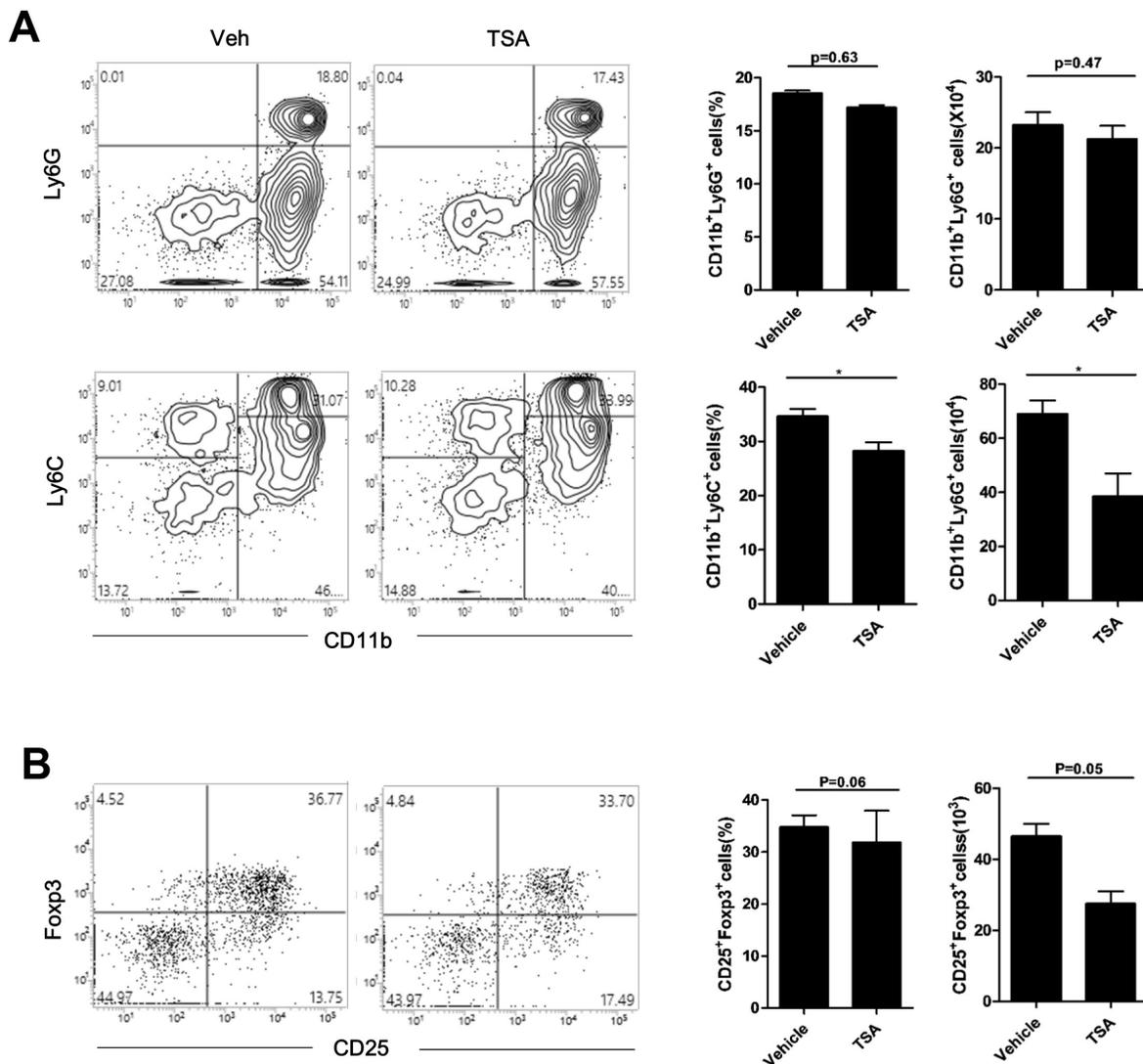


Trans-scirpusin A showed antitumor effects via autophagy activation and apoptosis induction of colorectal cancer cells

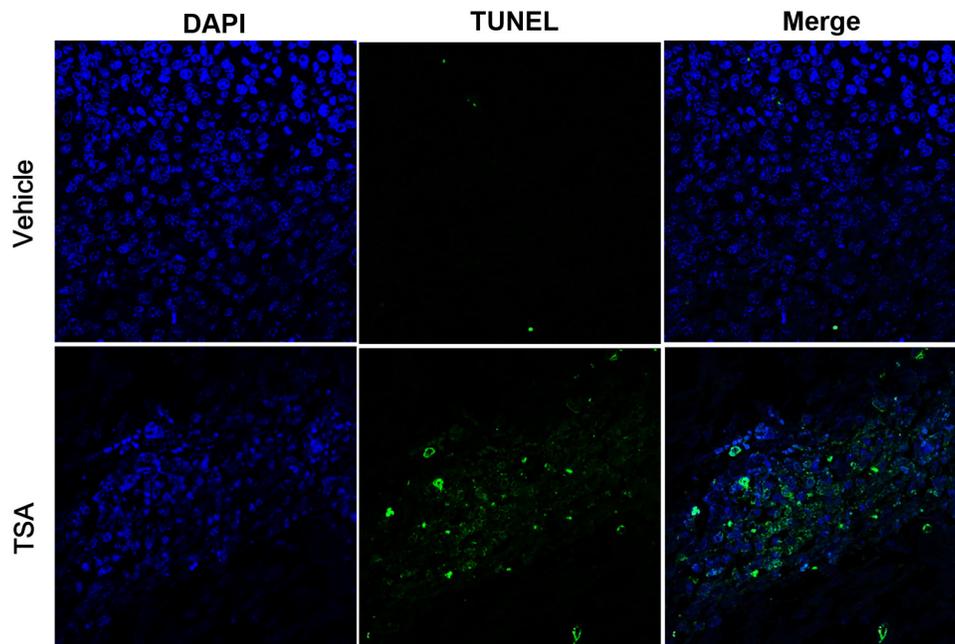
SUPPLEMENTARY MATERIALS



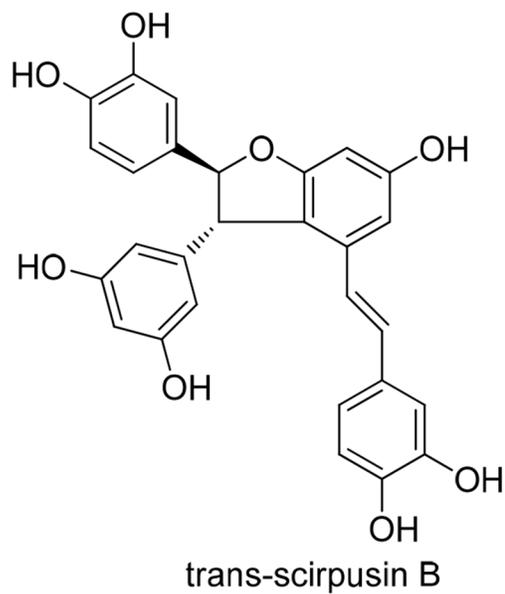
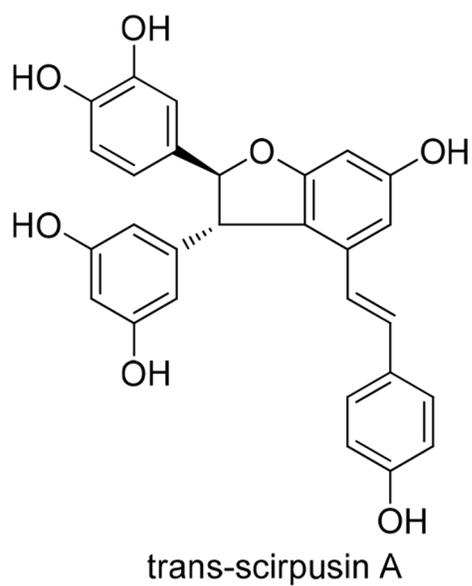
Supplementary Figure 1: Number of CD4⁺ T cell in spleen treated with TSA. Mice received s.c. injections of Her2/CT26 cells and were treated with TSA or vehicle. CD4⁺ T cells obtained from the spleens of Her2/CT26 tumor-bearing mice were analyzed by flow cytometry.



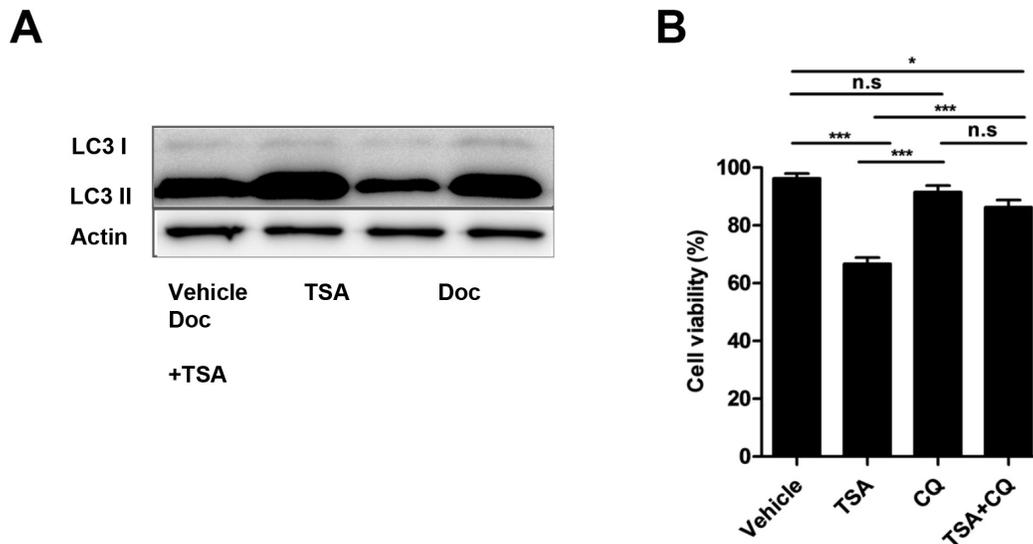
Supplementary Figure 2: TSA treatment could attenuate the immune-suppressive microenvironment in tumor tissue. Mice received s.c. injections of Her2/CT26 cells and were treated with TSA or vehicle. The MDSCs and Treg cells in tumor tissue were analyzed by flow cytometry after being gated by CD45 expression. **(A)** The percentage and number of CD11b⁺Ly6G⁺ and CD11b⁺Ly6C⁺ cells are shown (Two-tailed unpaired *t*-test). **(B)** For the determination of CD4⁺ Treg cells in the tumor tissue, the Foxp3⁺CD25⁺ gated population among CD4⁺ T cells was analyzed (Two-tailed unpaired *t*-test).



Supplementary Figure 3: TSA-induced apoptotic cell death in tumor tissue. BALB/c mice received *s.c.* injections of 2×10^5 Her2/CT26 tumor cells and TSA was started to be given by intraperitoneally (10mg/kg) for 3 times per every week as the average tumor volume reached 100 mm^3 for 2 weeks. The isolated tumor tissue and stained it using TUNEL assay. Apoptotic cells were examined by confocal microscopy.



Supplementary Figure 4: The Structure of trans-scirpusin A (TSA) and trans-scirpusin B. trans-scirpusin B has one more hydroxyl group in the phenyl ring as compared with the structure of trans-scirpusin A. Hence, this structural difference seems to make difference in bioactivity.



Supplementary Figure 5: TSA-induced autophagy activation and apoptotic cell death. (A) Her2/CT26 cells were treated TSA or both docetaxel (Doc) and TSA. The conversion of LC3-I to LC3-II was assessed using Western blots. (B) Her2/CT26 cells were treated TSA or both chloroquine (CQ) and TSA. Cell viability was evaluated at 24 h after treatment and was determined based on the absorbance at 480 nm. (*p<0.05, **p<0.01, and***p<0.0001, ns; not significant, ANOVA).