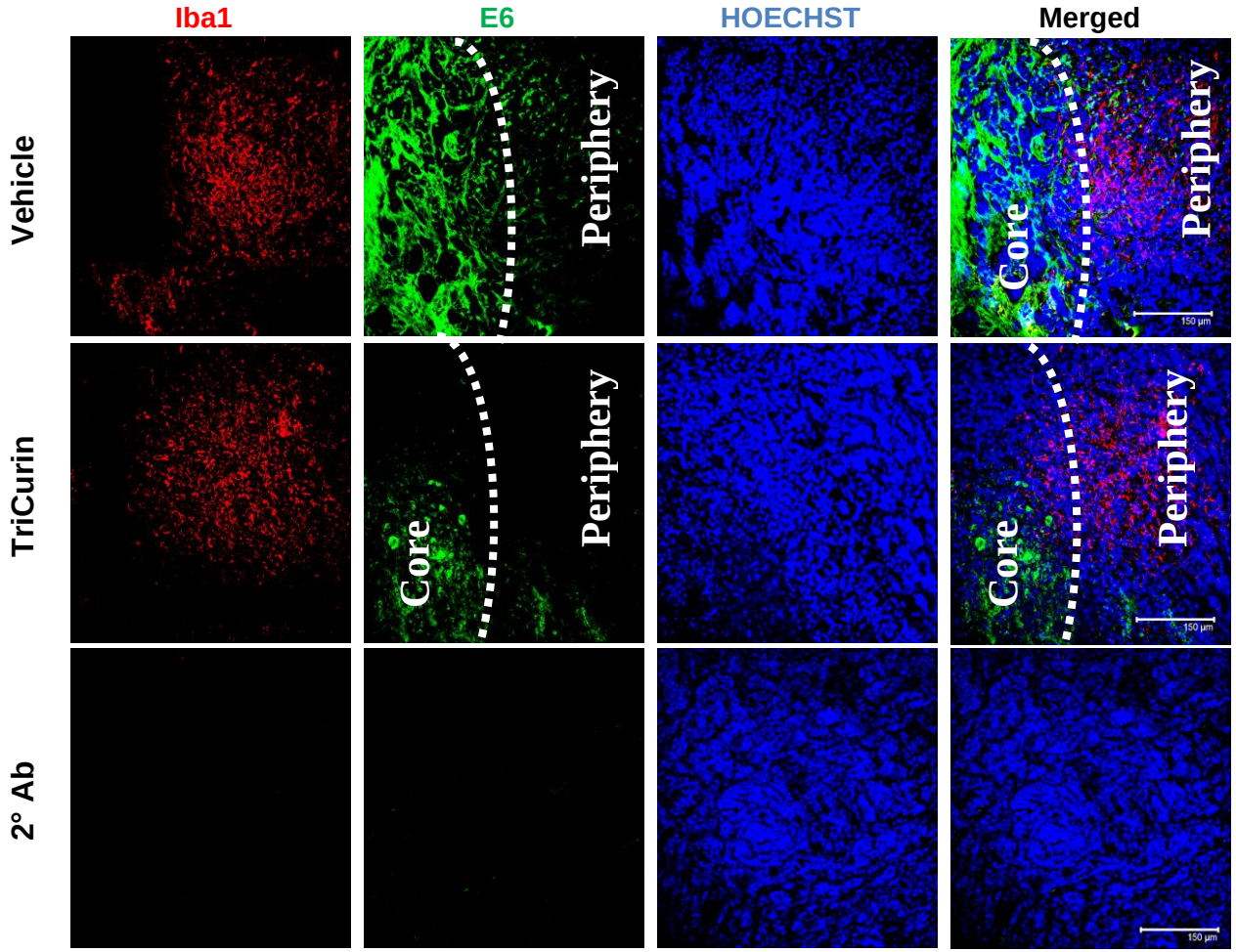
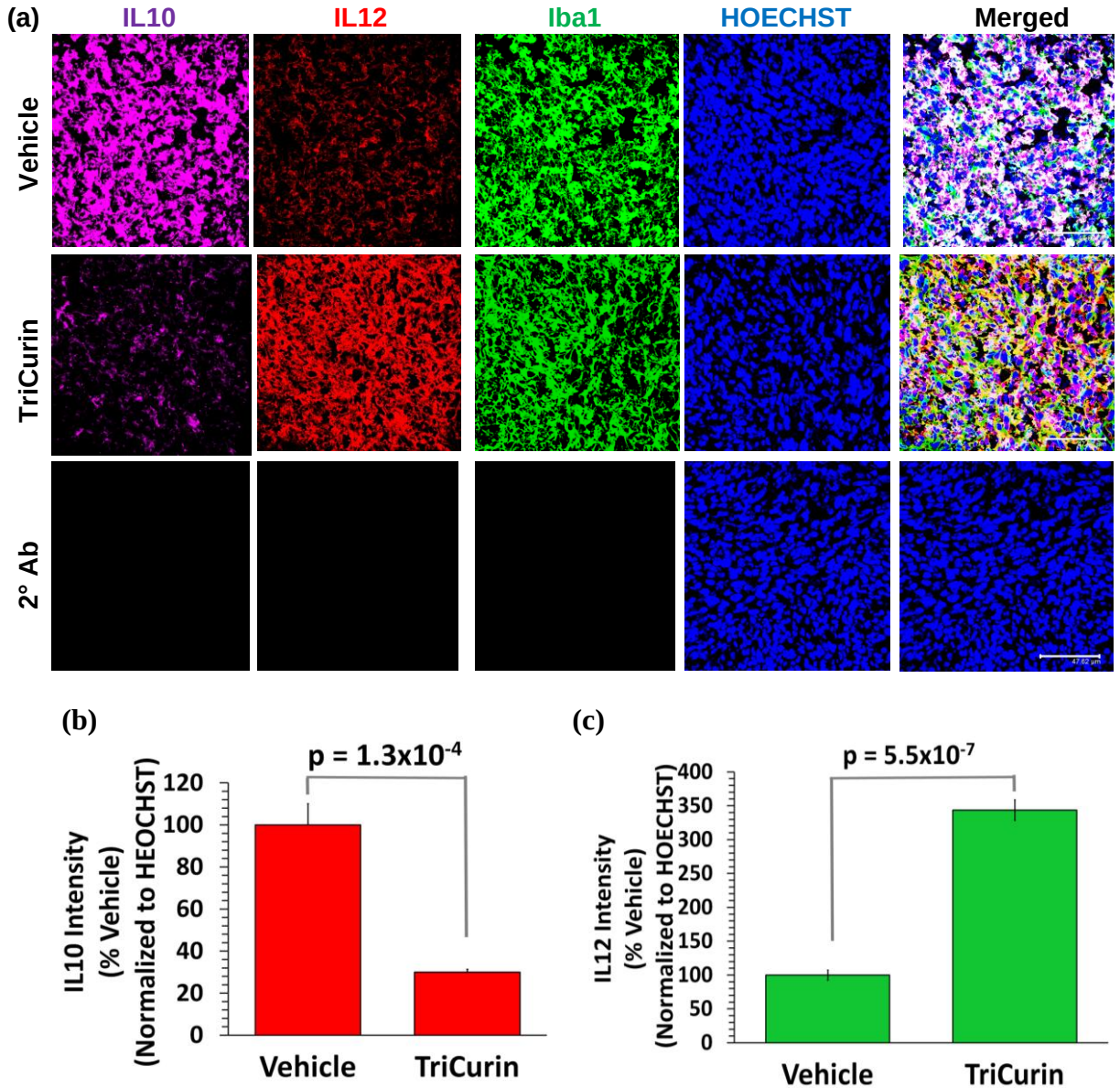
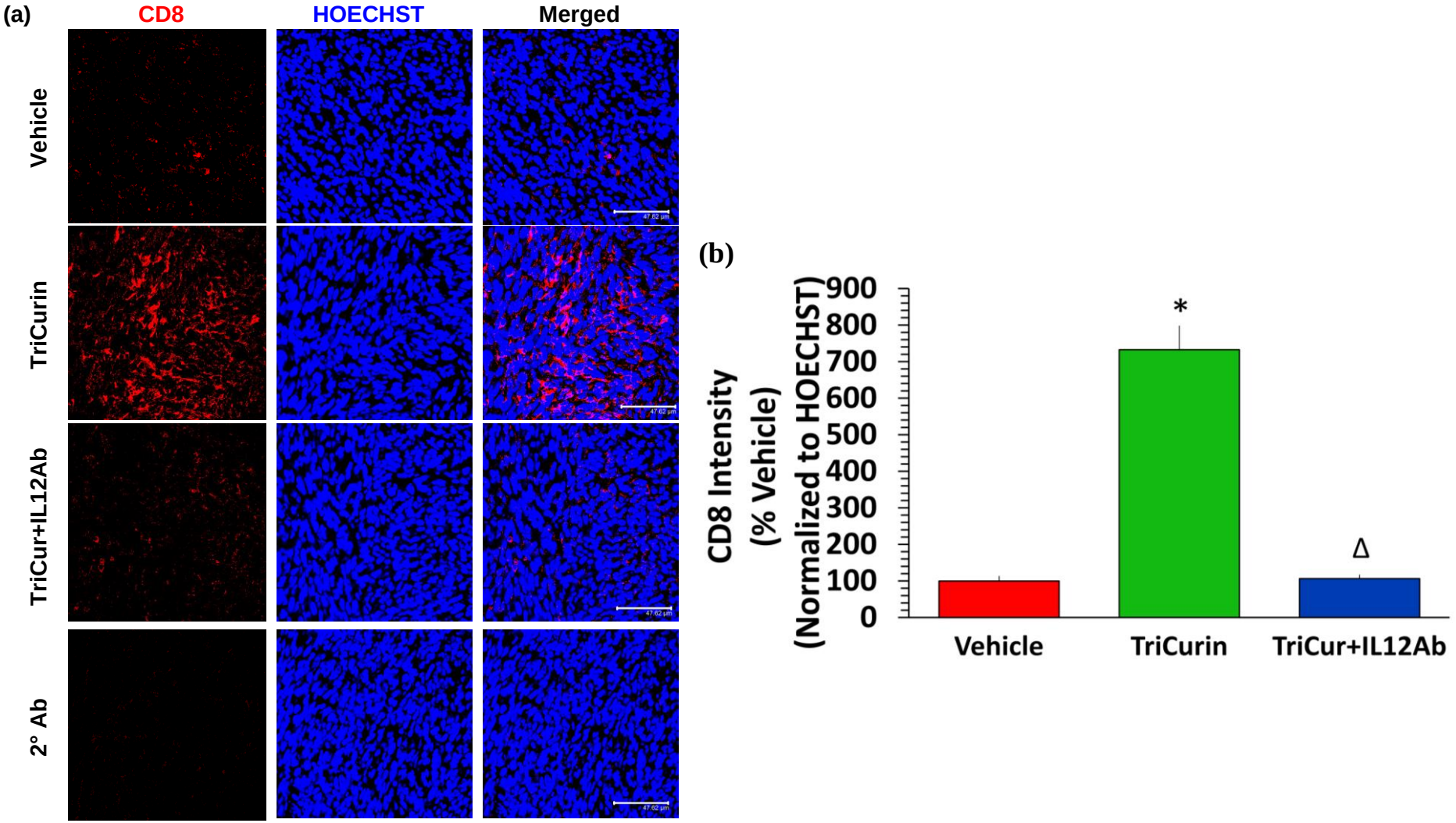


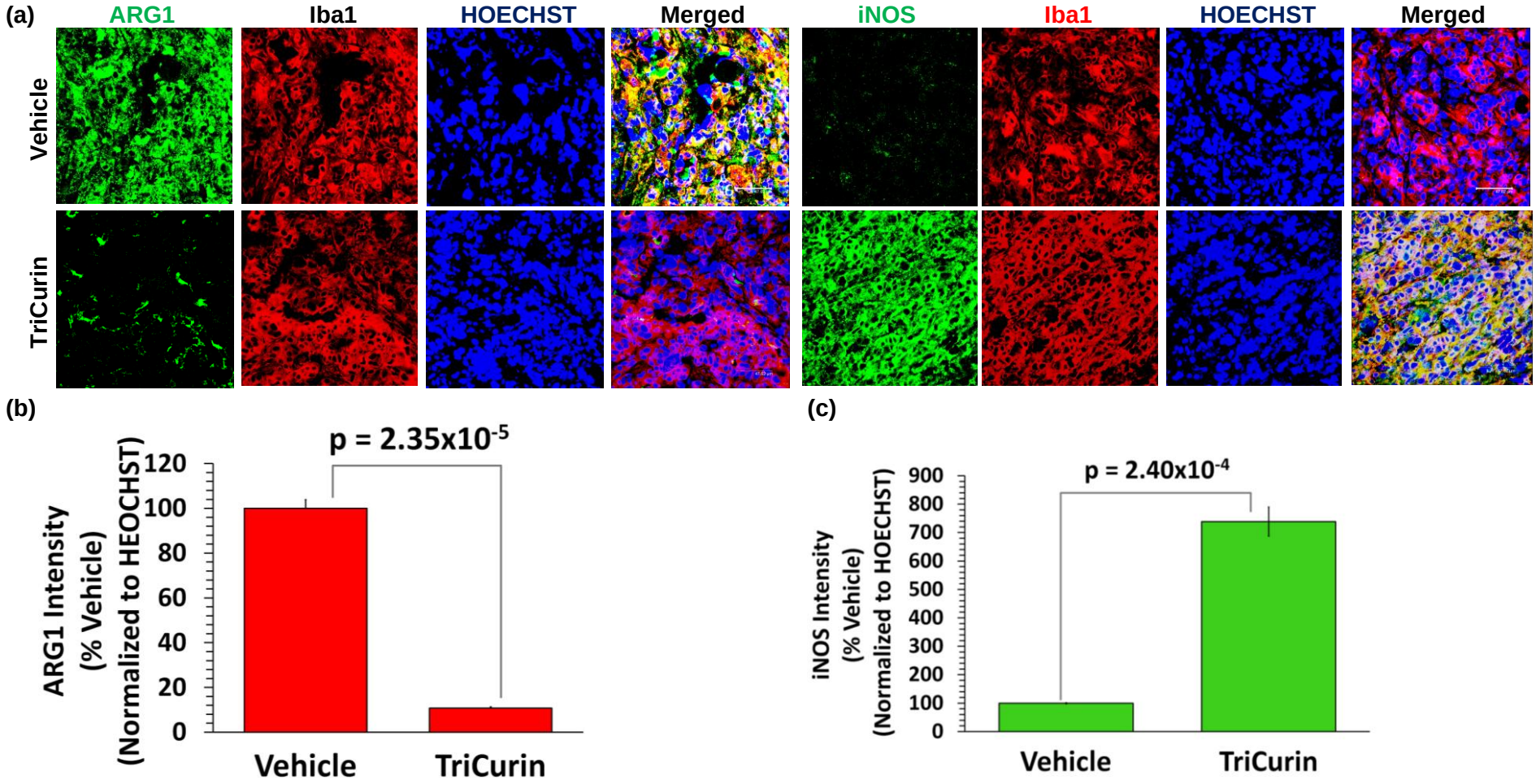
Supplementary Figure 1. Distribution of E6+ tumor cells and Iba1+ macrophages in tumor sections. Representative immunohistochemistry images (at 20X magnification) from tumors in the control **(upper row)** and TriCurin-treated **(middle row)** groups are shown. Tissue samples from one-half of TC-1 tumors from Vehicle and TriCurin groups were used to generate dispersed tumor cells for **Figure 1.** The remaining tissue samples were fixed, sectioned, and used for immunostaining with antibodies against E6 (tumor cells) and Iba1 (activated macrophage)**.** Tumors from TriCurin-treated mice showed less E6 staining of the tumor cells (green) in the tumor-core area. The tumor-periphery area in both groups harbored Iba1+ tumor-associated macrophages (TAM) (red). HOECHST = HOECHST33342. (Scale bar: 150 µm). **(Lower Row)** Lack of non-specific staining from the 2° antibodies.



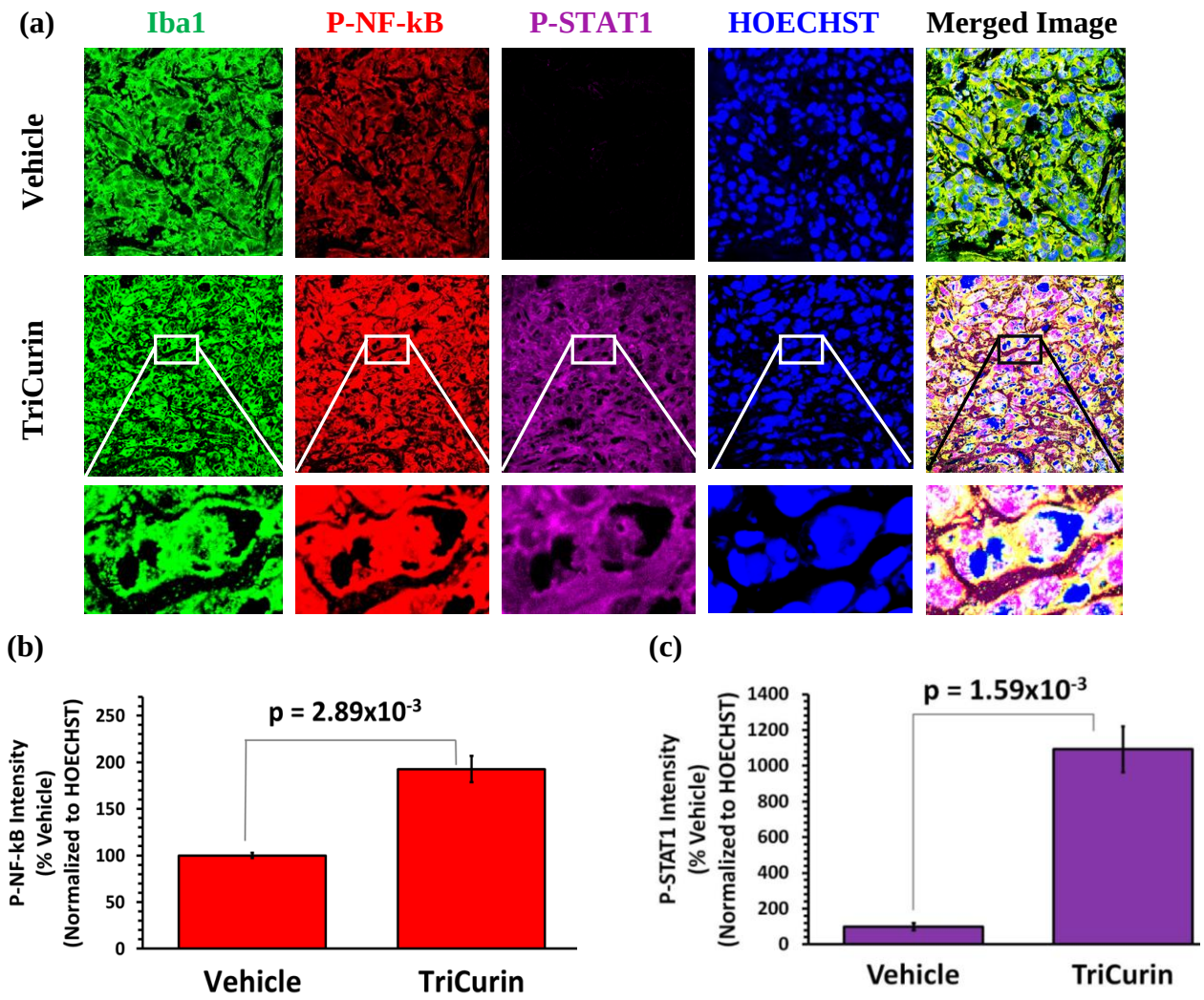
Supplementary Figure 2. Tumor-associated activated (Iba1+) macrophages (TAM) are IL10^{high} and IL12^{low}, whereas the TAM in the TriCurin-treated tumors are IL10^{low} and IL12^{high}. TC-1 Tumor sections from the Vehicle-treated and TriCurin-treated groups were triple-stained with Iba1, IL10, and IL12 antibodies. The TAM in Vehicle-treated display high levels of IL10 and low levels of IL12 **(a, upper row)**, whereas the tumors from the TriCurin-treated mice display IL10^{low} and IL12^{high} TAM **(a, middle row). (b and c).** The graphs show a 70% decrease in IL10 and a 244% increase in IL12 (mean ± S.E.M.) following TriCurin-treatment (n = 4 per group, with four randomly chosen tumor sections from each mouse). (Scale bar: 47.62 µm) (63X). **(a, lower row)** Lack of non-specific staining from the 2° antibodies.



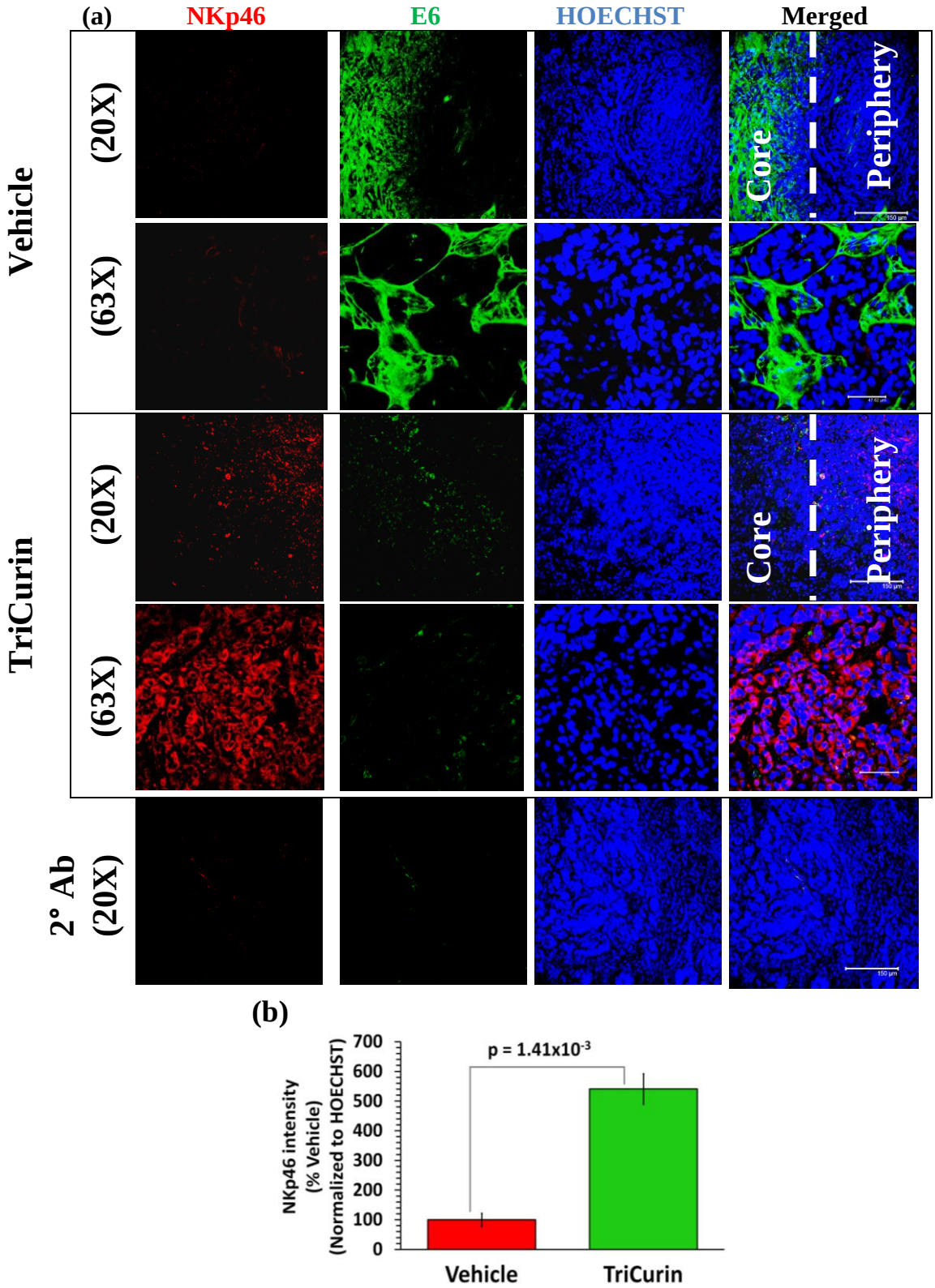
Supplementary Figure 3. IL12Ab treatment annulled TriCurin-evoked M1 TAM-mediated infiltration of Cytotoxic T lymphocytes (CTL) into TC-1 tumors. (a, first, second and third rows) and **(b)** TC-1 tumor sections from Vehicle-treated, TriCurin-treated and TriCurin+IL12Ab-treated mice were single-stained with the CD8 (CTL marker) antibody. The Vehicle group displays very little CD8 staining, whereas the TriCurin group shows a 633% increase in CD8 fluorescence (relative to Vehicle) (*$p = 7.3 \times 10^{-5}$), thus confirming the recruitment of activated CTL. TriCurin+IL12Ab treatment reversed this recruitment of CD8+ CTL to the level in the Vehicle group ($\Delta p = 7.4 \times 10^{-5}$ relative to TriCurin-treated). Four sections per mouse were used for imaging and the data (mean ± S.E.M.) obtained from Vehicle-treated (n = 4), TriCurin-treated (n = 4), and TriCurin+IL12Ab (n = 3) mice. (Scale bar: 47.62 µm) (63X). **(Fourth row)** Absence of non-specific staining from the 2° antibody.



Supplementary Figure 4. TriCurin induces M2 to M1 re-polarization of TAM in patient-derived head-and-neck Squamous Cell Carcinoma (HNSCC) xenografts. Tumors generated from HPV16+ human HNSCC UMSCC47 cells implanted into the flanks of athymic *nu/nu* (NCr) mice (xenograft) were injected with TriCurin (final estimated intra-tumor concentration ~64 µM+) for 5 weeks (3 doses per week). Parallel tumor sections were triple-stained to determine the levels of iNOS, ARG1, and Iba1 (marker for activated macrophages). **(a upper and lower rows)** Vehicle-treated tumor tissues display weak iNOS staining and strong ARG1 staining in the Iba1+ TAM. In contrast, the TriCurin-treated mice display an 89% decrease in ARG1 **(b)** and a 684% increase in iNOS **(c)**. Four sections per mouse were used for imaging and the graphical data were obtained from Vehicle-treated (n = 3), and TriCurin-treated (n = 3) mice. (63X) (Scale bar: 47.62 µm).



Supplementary Figure 5. TriCurin treatment causes co-localized activation of NF-kB and STAT1 in HNSCC xenograft tumor-associated macrophages. Parallel tumor sections from the experiment shown in **Supplementary Figure 4** were immunostained. **(a, middle and lower rows)** The P-Tyr^{701}-STAT1 (activated) and NF-kB p65 (P-Ser^{276}-p65) (activated) in the tumors are colocalized in the Iba1(+) cells. **(a-c)** The Vehicle-treated mice display basal levels of activated STAT1 and P-NF-kB p65. The TriCurin-treated mice show elevated levels of co-localized P-NF-kB (p65) (93% increase) and activated STAT1 (993% increase) in the Iba1+ TAM. Three sections per mouse were used for imaging and the graphical data were obtained from Vehicle-treated (n = 3), and TriCurin-treated (n = 3) mice. (Scale bar: 47.62 µm) (63X).



Supplementary Figure 6. TriCurin suppresses E6+ tumor cells and induces infiltration of natural killer (NK) cells into the HNSCC xenograft tumors. Parallel tumor sections from the experiment shown in **Supplementary figure 4** were double-stained with the NKp46 and E6 antibodies. **(a) first and third row (20X magnification); second and fourth row (63X magnification)** The Vehicle-treated tumor tissue displays minimal NKp46 stain, but the TriCurin-treated mice show a 441% increase in NKp46-staining (relative to Vehicle) (mean ± S.E.M.) **(b)**, thus confirming the recruitment of activated NK cells into the tumor. High, concomitant E6 expression observed in the Vehicle-treated sections was significantly suppressed after TriCurin-treatment [1]. Three sections per mouse were used for imaging and data were graphically presented from Vehicle-treated (n = 3), and TriCurin-treated (n = 3) mice. (Scale bar: 150 µm for 20X) (Scale bar: 47.62 µm for 63X). **(a, fifth row)** Lack of non-specific stainining from the 2° antibodies**.**

References:

1. Piao L, Mukherjee S, Chang Q et al. (2017) TriCurin, a novel formulation of curcumin, epicatechin gallate, and resveratrol, inhibits the tumorigenicity of human papillomavirus-positive head and neck squamous cell carcinoma. Oncotarget. 8: 60025-35. doi: 10.18632/oncotarget.10620