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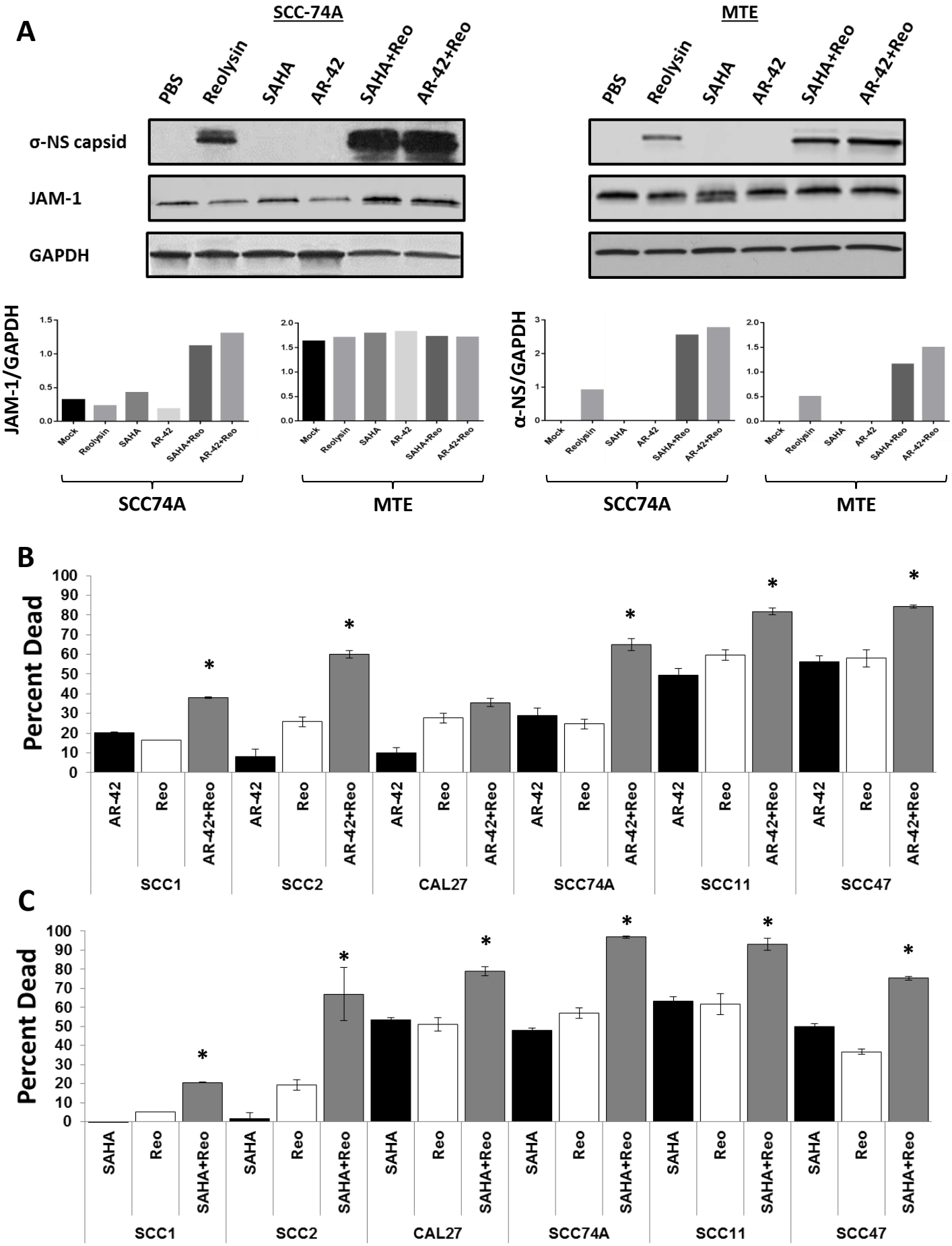
Supplemental Information

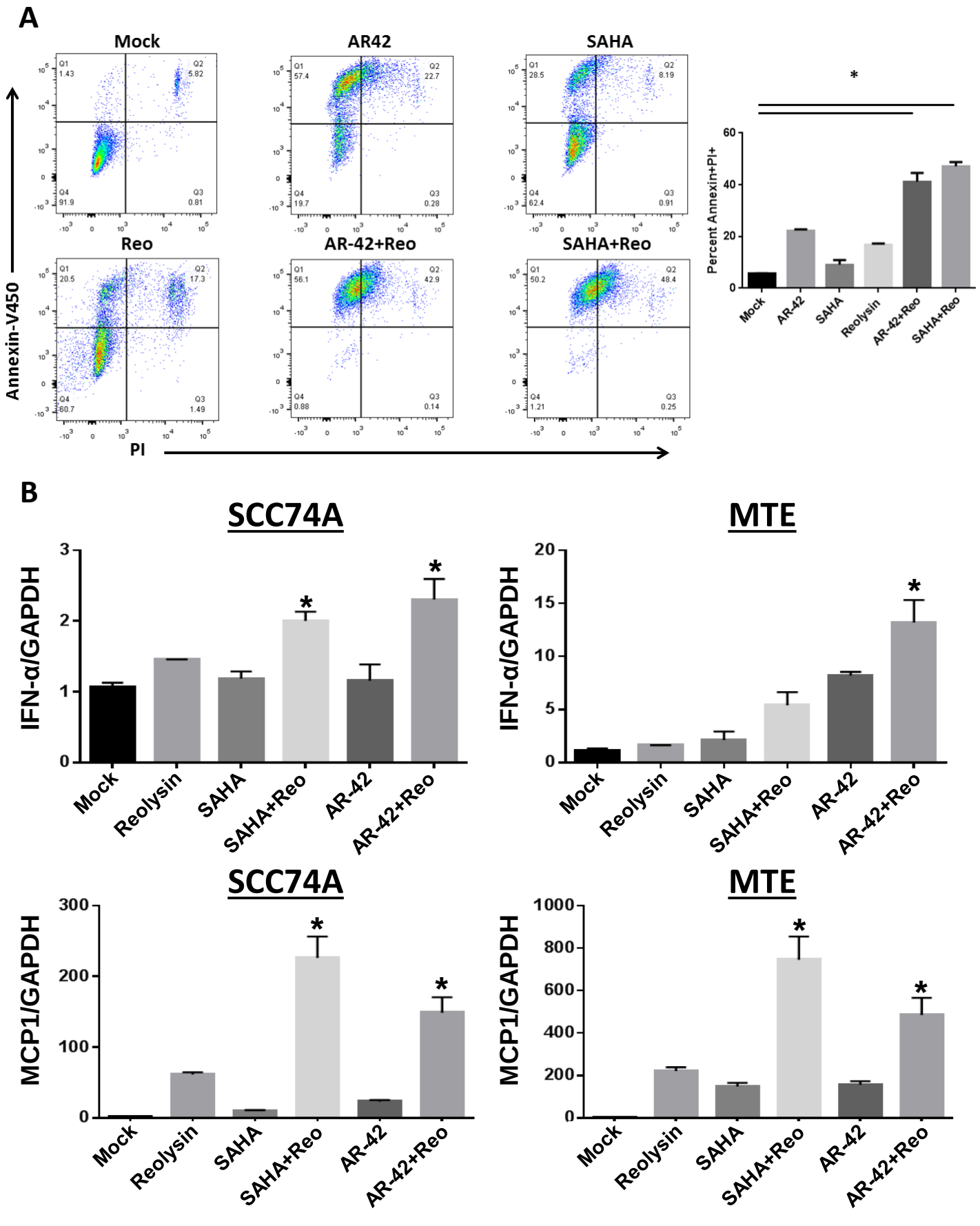
Reolysin and Histone Deacetylase Inhibition in the Treatment of Head and Neck Squamous Cell Carcinoma

Alena C. Jaime-Ramirez, Jun-Ge Yu, Enrico Caserta, Ji Young Yoo, Jianying Zhang, Tae Jin Lee, Craig Hofmeister, John H. Lee, Bhavna Kumar, Quintin Pan, Pawan Kumar, Robert Baiocchi, Theodoros Teknos, Flavia Pichiorri, Balveen Kaur, and Matthew Old

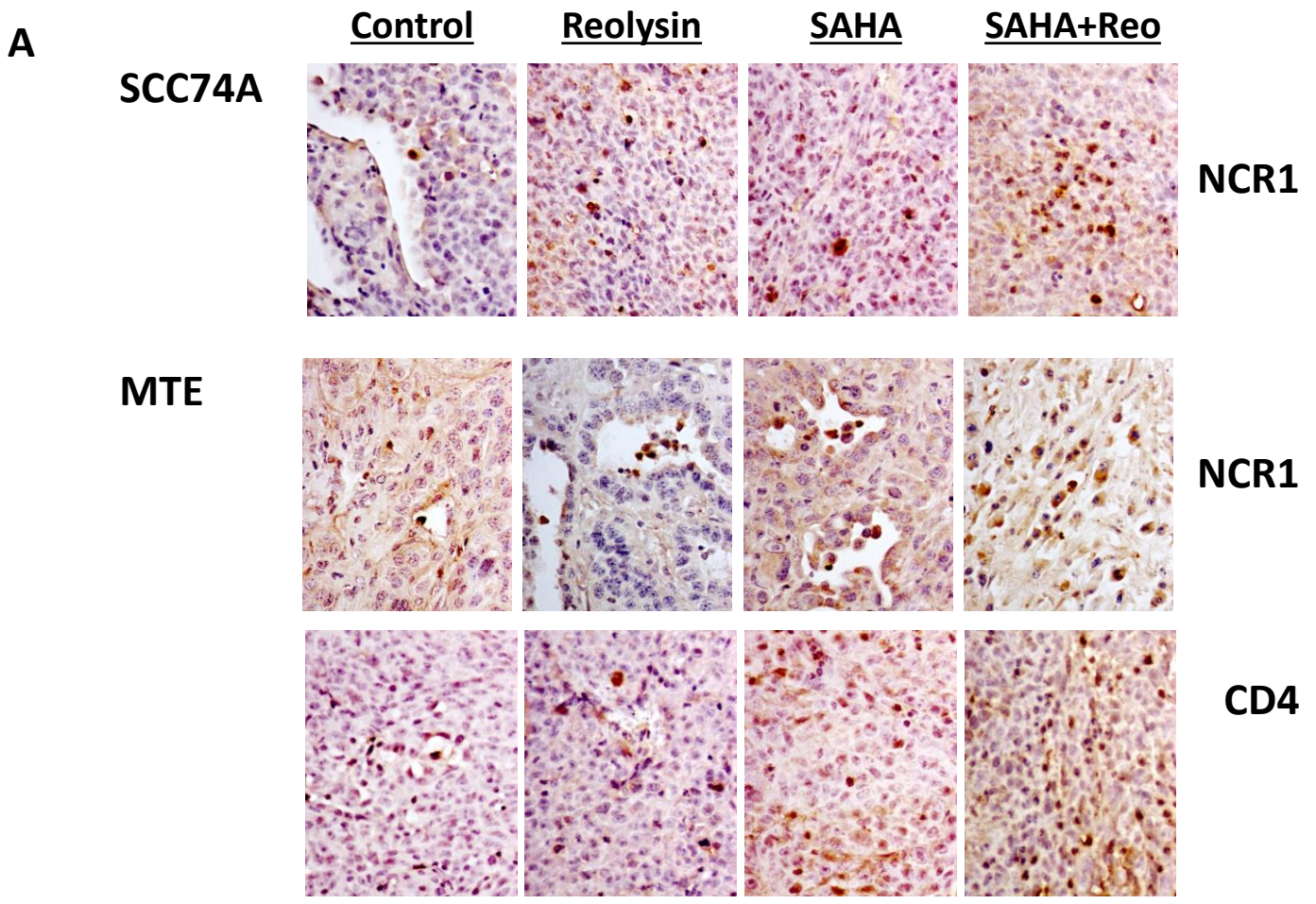
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 1. Combinatorial HDACi and Reolysin treatment enhances JAM-1 and reovirus capsid protein expression. A. The levels of the JAM-1 entry receptor and Reovirus capsid protein (σ-NS) were assessed in SCC-74A human (left panel) and MTE murine (right panel) squamous carcinoma cells. Cells were treated with AR-42 (10 μM) or SAHA (20 μM) followed by Reolysin (10 MOI) for 48 hours, collected and subjected to Western Blot analysis. Quantification of JAM-1 or σ-NS normalized to GAPDH for each cell line is indicated below. The impact of **B.** AR-42 or **C.** SAHA and Reolysin combinatorial therapy was assessed on human SCC-1, SCC-2, Cal27, SCC-74A, SCC-11 and SCC-47 head and neck cancer cell lines following 48 hours of treatment via a standard MTT assay. *indicates combination treatment differences compared to each individual treatment group with $p<0.05$. All experiments were performed in triplicate.

Supplemental Figure 2. SAHA plus Reolysin treatment enhances head and neck cancer cell killing, apoptosis and inflammatory responses. A. Murine MTE squamous carcinoma cells were treated with AR-42 (10 μM) or SAHA (20 μM) and/or 10 MOI of Reolysin for 48 hrs to assess cellular apoptosis V flow cytometric analysis for propidium iodide (PI) and annexin-V450. Representative PI/annexin scatter plots with quantification to the right (n=3/group). *indicates combination treatment differences compared to each individual treatment group with $p\leq0.01$. **B.** Human SCC74A were treated with SAHA (20 μM), AR-42 (10 μM) and/or Reolysin (10 MOI) for 24 hours. Murine MTE were treated with SAHA (10 μM), AR-42 (5 μM) and/or Reolysin (5 MOI) for 24 hours. Cells were then collected and RNA was harvested for qPCR analysis of IFN-α and MCP-1. *indicates

combination treatment differences compared to each individual treatment group with $p<0.05$. All experiments were performed in triplicate.

Supplemental Figure 3. Immune cell tumor infiltrate and circulating populations. A. Representative NK cell (NCR1) for both SCC74A and MTE stained tumors, as well as T cell (CD4) IHC in MTE tumors at the time of death when tumors reached ~1500 mm^3 at a magnification of 400X. **B.** Flow cytometric analysis of whole splenocytes isolated from C57BL/6 mice bearing subcutaneous MTE squamous carcinoma cells and treated with SAHA, Reolysin, or the combination indicated (n=3 mice/group) at seven days post tumor recruitment. Immune cells were phenotyped using antibodies against activated NK cells (CD49b+CD335+), myeloid derived suppressor cells (CD11b+Gr1+), macrophages (CD11b+F4/80+), CD4+ T cells, CD8+ T cells, effector CD4+ or CD8+ T cells (CD4+ or CD8+ and CD3+CD44+CD62L+), or dendritic cells (CD11c+ and CD80+ or MHCII+). Quantification revealed no significant differences between treatment groups. Murine studies were performed in duplicate.