Background
CAR T cell therapy has greatly improved outcomes in relapsed hematologic malignancies, yet some patients do not respond to treatment. Dysregulation of apoptotic signaling pathways has been implicated in reduced sensitivity to chemotherapeutics in many hematologic malignancies, however, whether these pathways are also perturbed as a mechanism of resistance to CAR T cell therapy remains unclear. Here, we investigated the importance of mitochondrial apoptosis in CAR T cell anti-tumor cytotoxicity.

Methods
Given that knockout of the pro-apoptotic proteins Bak and Bax is sufficient to significantly dampen mitochondrial apoptosis, we generated HeLa, HCT-116, JeKo-1 and NALM6 cells lacking Bak and Bax and cultured them with CAR T cells in order to determine the relevance of mitochondrial apoptosis to CAR T cell cytotoxicity. Cytoxicity was measured by Annexin V/Hoechst staining, impedance assays and colony formation assays.

Results
HeLa cells expressing CD19 and lacking Bak and Bax (HeLa-19-DKO) exhibited significantly enhanced resistance to killing by CD19 CAR T cells when compared to cells expressing endogenous levels of Bak and Bax. Given the protection from CAR T cell cytotoxicity conferred by loss of the pro-apoptotic proteins Bak and Bax, we next conversely sought to determine if overexpression of the anti-apoptotic proteins Bcl-2 and Bcl-XL would similarly confer protection. Consistent with our findings using DKO cells, HeLa-19 cells overexpressing Bcl-2 and Bcl-XL were more resistant to killing by CD19 CAR T cells, demonstrating that mitochondrial apoptosis is relevant to CAR T cell cytotoxicity.

We next sought to validate the importance of mitochondrial apoptosis in CAR T cell cytotoxicity by targeting an endogenously expressed CAR T cell target, namely EGFR. Coculture of EGFR CAR T cells with wild type and DKO HeLa and HCT-116 cells revealed significant resistance to CAR T cells by DKO target cells. Given the protective effect of dampening mitochondrial apoptosis in two solid cancer models, we finally sought to determine whether CAR T cells similarly utilize mitochondrial apoptosis to induce cell death in liquid cancers. In contrast with our solid tumor models, knockout of Bak and Bax in the liquid cell lines JeKo-1 and NALM6 did not result in enhanced resistance to CD19 CAR T cell cytotoxicity.

Conclusions
Taken together, these data demonstrate that mitochondrial apoptosis plays a role in how CAR T cells kill solid but not liquid tumors, suggesting that cancer type may be an important factor in considering combinatorial treatments using CAR T cells and pro-apoptotic drugs.