Background: Macrophages can be repolarized to promote tumor destruction, particularly by triggering tumoricidal phagocytosis of cancer cells. Despite the increasing interest in monoclonal antibody therapies targeting tumor antigens to drive cancer cell elimination via phagocidal macrophages, we still face tremendous challenges in developing adequate therapeutics due to lack of knowledge of the signaling pathways that induce phagocidal macrophages. Our previous work showed that cIAP1/2 antagonism and T cell derived cytokines promote anti-tumor immunity by reprogramming tumoricidal macrophages to phagocytose live tumor cells.

Methods: Here we performed transcriptional analysis on both macrophages and tumor cells treated with cIAP1/2 antagonism or vehicle and identified candidate positive and negative regulators of phagocytosis. We are demonstrating how these receptors-ligand pairs on macrophages and tumor cells are induced and the mechanisms by which they effect tumor cell destruction. We also performed an in vitro CRISPR screen on tumor cells cocultured with macrophages, treated with cIAP1/2 antagonism or vehicle, recovered the DNA of phagocytosed tumor cells from within macrophages and compared to non-phagocytosed tumor cells to discover mediators of resistance or sensitivity to both baseline phagocytosis (efferocytosis) and cIAP1/2 antagonism induced phagocytosis. Our screen revealed a striking lack of dependence on MHC class I for tumors treated in vivo. Genes involved in phagocytosis resistance instead converged on regulation of the cytoskeleton and other candidates which we have now validated in single gene knockout tumor cells and will be discussed. This study will provide critical insights on tumoricidal macrophages, uncover the key pathways reprogramming these myeloid cells, and decipher the essential tumor-intrinsic pathways resulting in sensitivity and resistance to anti-tumor phagocytosis.