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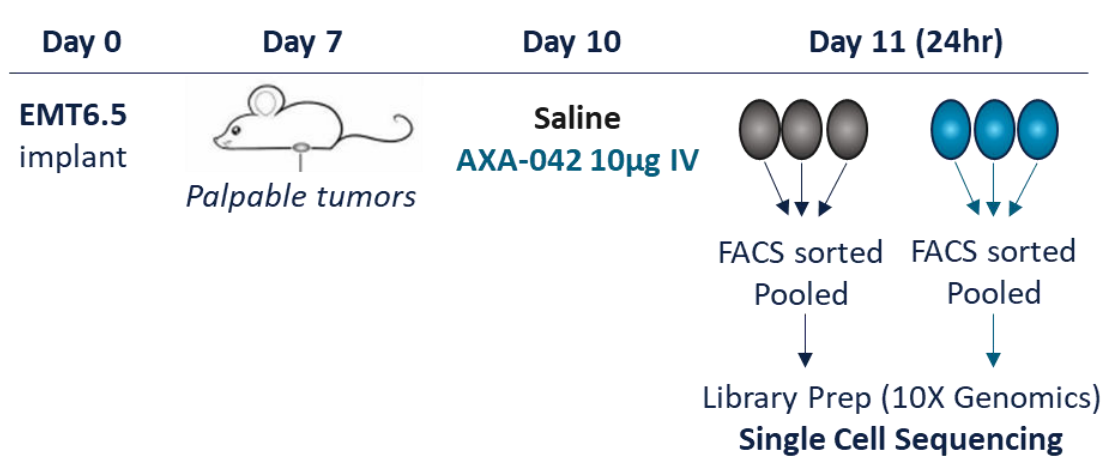


## INTRODUCTION

- Treatment approaches that engage both the innate and adaptive immune response have the potential to transform anti-cancer therapy, especially in settings of checkpoint inhibitor insensitivity or acquired resistance.
- Toll-like receptors (TLRs) mediate the initial cellular response to external pathogens or endogenous alarmins, activating downstream pro-inflammatory cascades and leading to the activation and recruitment of key innate subsets.
- TLR2 is a cell surface receptor, expressed on macrophages, dendritic cells (DC), neutrophils and subsets of NK and T cells. TLR2 signaling plays an important role in NK and T cell cytolytic cell functions, as well as DC and macrophage activation.
- AXA-042 is a novel synthetic TLR2/6 agonist designed for systemic delivery to re-engage the innate immune response to help overcome tumor immune escape.
- Once-a-week treatment with AXA-042 led to 87% growth inhibition of syngeneic EMT6 tumors (AACR Annual Meeting 2022 Poster 3502)

## MATERIALS AND METHODS

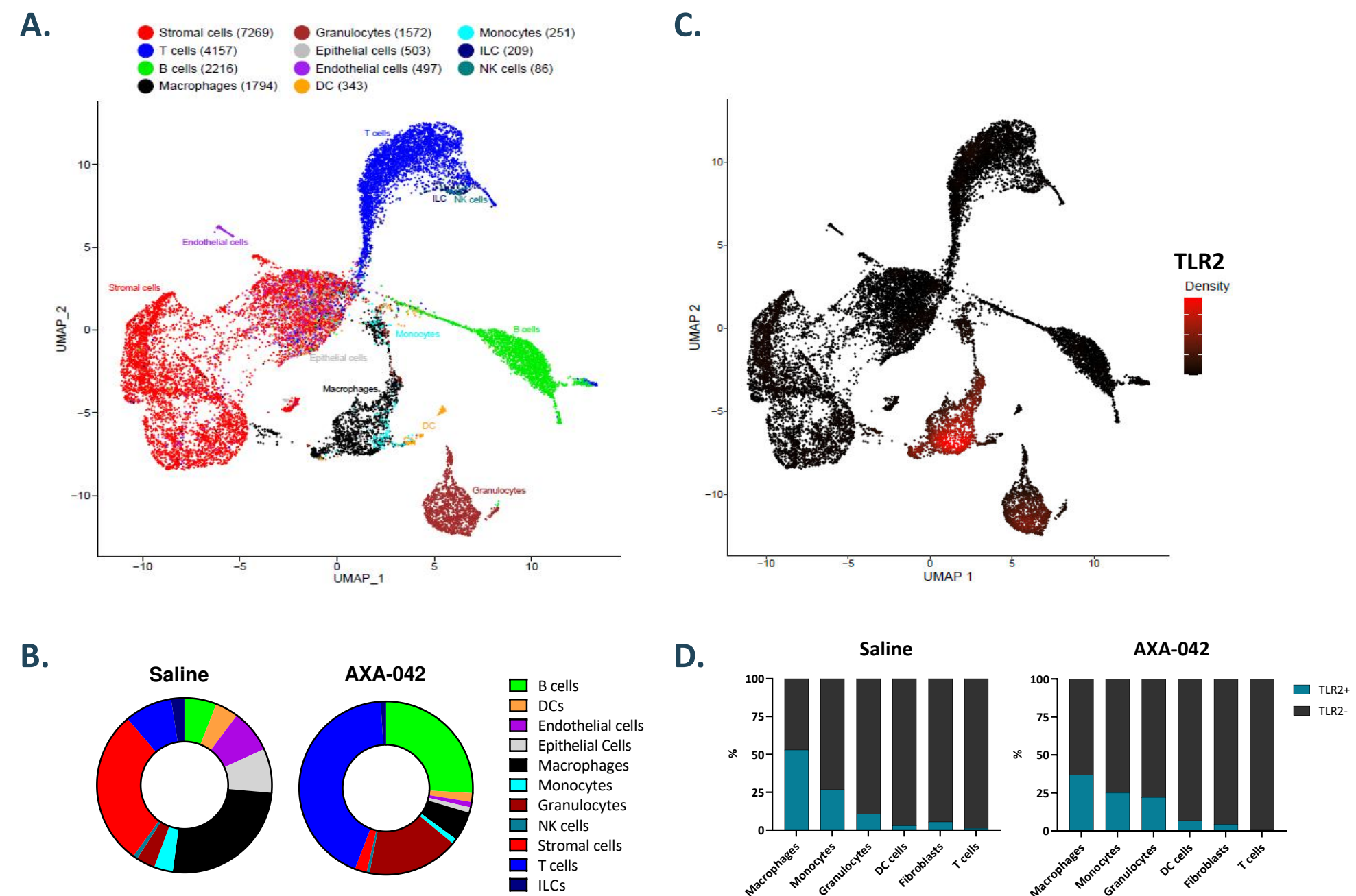
- Female Balb/c mice bearing orthotopic EMT6.5 mammary tumors received a single treatment of saline or AXA-042 (10 µg/mouse IV)
- Equal numbers of viable cells were recovered from collagenase-digested tumors (n=3/group) 24 hours after treatment and pooled prior to loading onto a Chromium Single Cell Chip



- 10x Genomics single-cell transcriptome libraries were prepared from each sample and sequenced with Illumina NextSeq 550 sequencing platform.
- Cell type annotation based on the ImmGen database was determined using the SingleR package. T cell subsets were defined using TILPRED.
- Differential gene expression analyses were completed to identify AXA-042 responsive gene signatures in cell subsets that included at least 10 cells. The identified genes were required to be expressed in at least 3 cells.

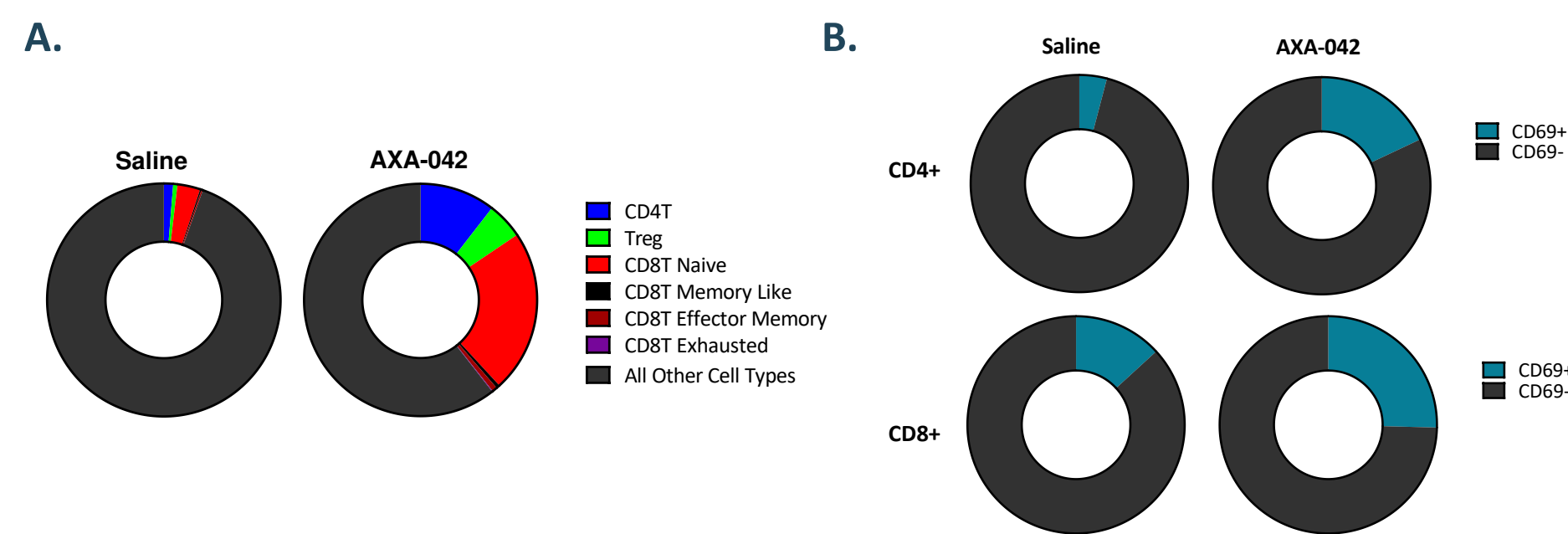
## RESULTS

Figure 1. AXA-042 Treatment Alters the Composition of the EMT6.5 Tumor Environment



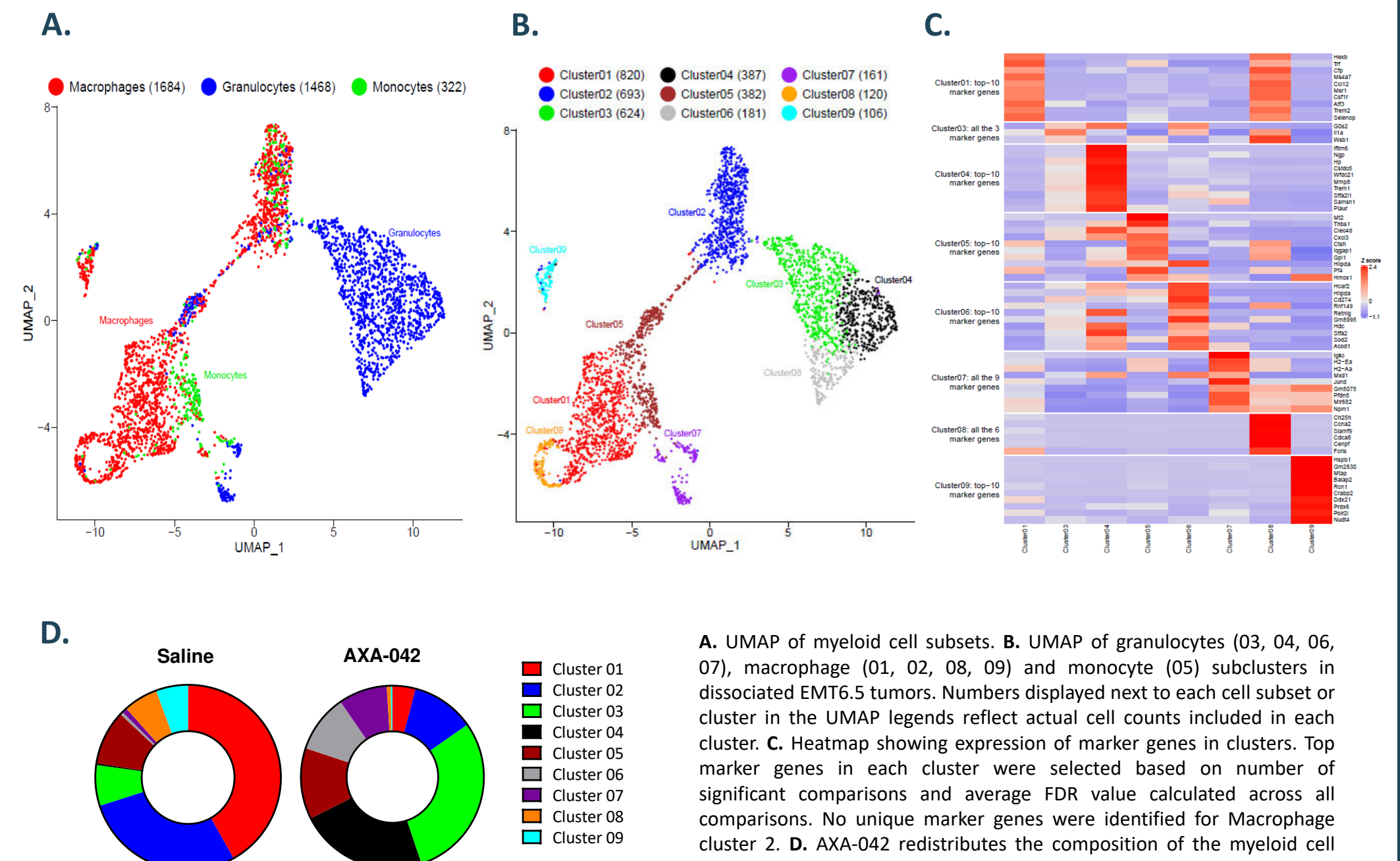
A. Single-cell transcriptomes for all cells dissociated from EMT6.5 tumors, visualized on a Uniform Manifold Approximation and Projection (UMAP) plot. Numbers displayed next to each cell type in the legend reflect actual cell counts included in each cluster. B. AXA-042 treatment redistributes the cell composition of the EMT6.5 tumor. Data is shown as % of each cell type. C. UMAP plot demonstrating enrichment of TLR2 expression in macrophages, neutrophils and dendritic cell subsets. D. Impact of AXA-042 treatment on TLR2 expression in indicated subsets. Data is shown as % of each cell type.

Figure 2. AXA-042 Increases Proportion of Activated T Cell Subsets in EMT6.5 Tumors



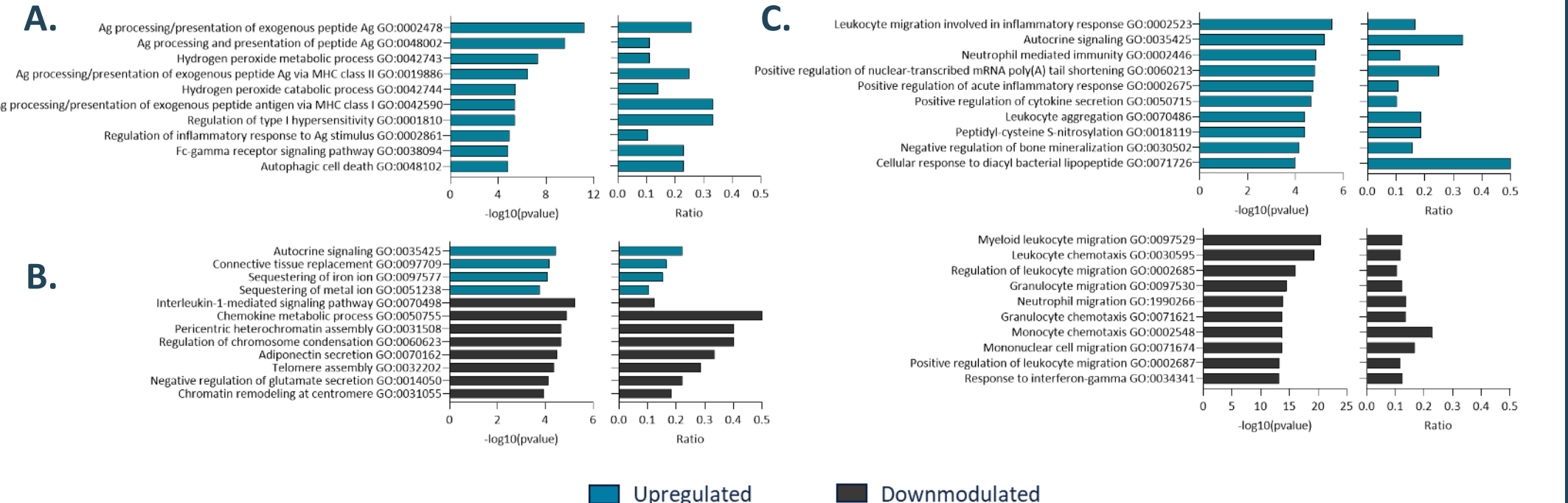
A. Impact of AXA-042 treatment on proportion of T cell subsets in EMT6.5 dissociated tumors. Data is shown as % of each cell type relative to all tumor cells. B. AXA-042 increases proportion of activated CD69+ CD4+ and CD8+ T cells 24hr post treatment. Data is shown as % of each cell type.

Figure 3. AXA-042 Alters the Composition of Myeloid Population Subclusters



A. UMAP of myeloid cell subsets. B. UMAP of granulocytes (03, 04, 06, 07), macrophage (01, 02, 08, 09) and monocyte (05) subclusters in dissociated EMT6.5 tumors. Numbers displayed next to each cell subset or cluster in the UMAP legends reflect actual cell counts included in each cluster. C. Heatmap showing expression of marker genes in clusters. Top marker genes in each cluster were selected based on number of significant comparisons and average FDR value calculated across all comparisons. No unique marker genes were identified for Macrophage cluster 2. D. AXA-042 redistributes the composition of the myeloid cell subclusters 24hr post treatment: decreasing macrophage sub-clusters 01, 02, 08 and 09 and increasing the neutrophil subsets 03, 04, 06 and 07.

Figure 4. GO Pathway Enrichment Analysis for Differentially Expressed Genes in TLR2+ Myeloid Subsets Post AXA-042 Treatment



A-C. Plots of the top enriched GO terms for differentially expressed genes in TLR2+ Monocytes (A), Granulocytes (B) and Macrophages (C). Significance set at p<0.001, data is shown as -log10(p value) or gene ratio (cut-off set at 10% of the GO term signature genes)

## CONCLUSIONS

- Systemic AXA-042 treatment led to a reorganization of the tumor microenvironment within 24 hours of treatment.
- AXA-042 altered the myeloid cell subset profiles, promoted the influx of activated T cells and reduced tumor cell viability.
- AXA-042 has completed GLP toxicology studies and is undergoing evaluation in a Phase 1 clinical trial (ACTRN12622000993796) in advanced solid tumors.