Dear Editors:

We would like to submit the enclosed manuscript entitled “Analysis of key cell interaction networks and dynamic regulation of tumor microenvironment in multi-stage evolution of lung adenocarcinoma based on single cell transcriptome sequencing”, which we wish to be considered for publication on CIR. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. We confirm that the manuscript is only submitted to CIR. We are not submitted to other magazines and not under consideration for publication elsewhere, in whole or in part. we confirm that all the research meets the ethical guidelines and adherence to the legal requirements of the study country. This manuscript was not previously submitted on CIR. All authors have made important contributions, and all authors agree with the content of the manuscript.

In this study, we performed scRNA-seq on tumor samples from seven LUAD patients, including three with adenocarcinoma in situ (AIS) and four with invasive lung cancer (ILC), to systematically delineate key macrophage subsets and epithelial cell interactions within the tumor microenvironment. By integrating multidimensional bioinformatics approaches—including cell-type composition analysis, differential gene expression, intercellular communication, pseudotime trajectory inference, and exosome functional profiling—we revealed distinct differentiation patterns of malignant alveolar type II epithelial cells (AT2), macrophage subsets, and exhausted CD4+/CD8+ T cells during tumor progression.Notably, we identified a macrophage subpopulation characterized by high expression of SPP1 (secreted phosphoprotein 1), classical myeloid markers (CD14, FCGR3A), and M2 polarization markers (CD163, MRC1, TREM2), which we termed SPP1⁺MDMs.M2. Functional assays and pathway enrichment analysis demonstrated that SPP1 acts as a central molecular hub driving M2 polarization of macrophages via activation of the PI3K signaling pathway, thereby promoting tumor proliferation, invasion, and metastasis. Furthermore, based on regulatory network analysis, we constructed an exosome-associated lncRNA/circRNA–mRNA regulatory network targeting SPP1, elucidating a potential mechanism by which non-coding RNAs modulate SPP1+MDMs.M2 differentiation.Collectively, our study provides a comprehensive framework for understanding the dynamic evolution of the LUAD immune microenvironment and highlights SPP1+MDMs.M2 as a potential target for precision immunotherapy.