**ReadMe**

This folder **Preprocessing** contains the code files used to preprocess the original data (in .fcs format) from the paper ***“Adjuvant dendritic cell-based immunotherapy after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with malignant peritoneal mesothelioma- a phase II clinical trial”.*** Mainly, for the three T-cell panels: Co-inhibition, Co-stimulation, and Cytokine. But, some preprocessing was also done on the two panels for B-cells and DCMyeloid, then was not completed because we focused on the T-cell panels only.

**Step 1:** The original .fcs files naming convention were not quite explanatory. So, we renamed the files and divided them into three subfolders (**TP1, TP2, and TP3**) for the three timepoints along the vaccination/ immunotherapy process. The renamed files are in .fcs format. **Code file: RenameFCSFiles.R**

**Step 2:** The generic preprocessing steps such as margin event removal, compensation, transformation, scaling, and quality control were done. The resulting .fcs files are in the three subfolders **PeacoQC\_TP1, PeacoQC\_TP2, and PeacoQC\_TP3**. **Code files:** **PeacoQC.R** (general code file), **MPM…Panel.R** (the … is the name of the panels)

**Step 3:** The quality controlled and preprocessed .fcs files were gated for Lymphocytes. The gated Lymphocytes were in separate .fcs files (coming from FlowJo) based on the gating quadrants, so they needed to be merged. The merged .fcs files are in the folder named **Lymphocytes TP1-2-3**. **Code file: MPMGate1Merge.R**

**Step 4:** The Lymphocytes are normalized in two steps. The cell type markers are normalized first and the .fcs files are kept in the folder **Normalized\_Cell\_Type\_Marker,** then the cell state markers are normalized and the .fcs fileskept in the folder **Normalized data. Code files: MPMTcellNormalize.R, MPMTcellNormalize\_2.R**

**Step 5:** Cell type assignment using any algorithm of choice. We have our semi-automated gating method that we used. The final data with the cell types are stored in .xlsx files in the folder **Final Cell Assigned Data**. **Code files: MPM…CellAssign.R**

\*More details on the data are available in the readme file in the **Data > Malignant Peritoneal Mesothelioma Dataset** folder.

**\*\*The data file paths in these codes can be old or path-dependent. Needs careful updating if you want to use them for your own data. We do not recommend running the preprocessing codes for the MPM dataset, because the data is already processed and stored.**