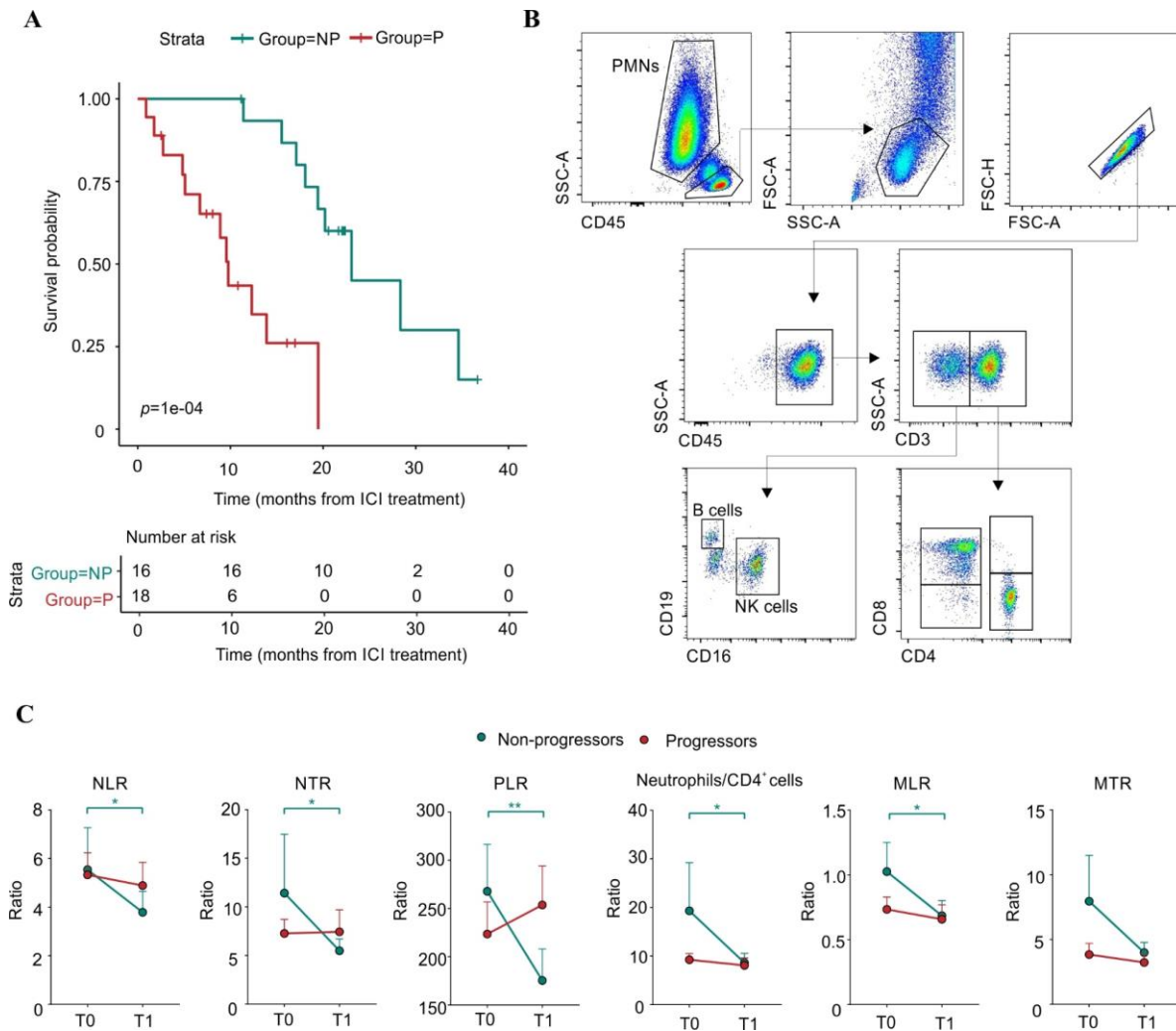


Supplementary material of manuscript ONCOIMM-20230220

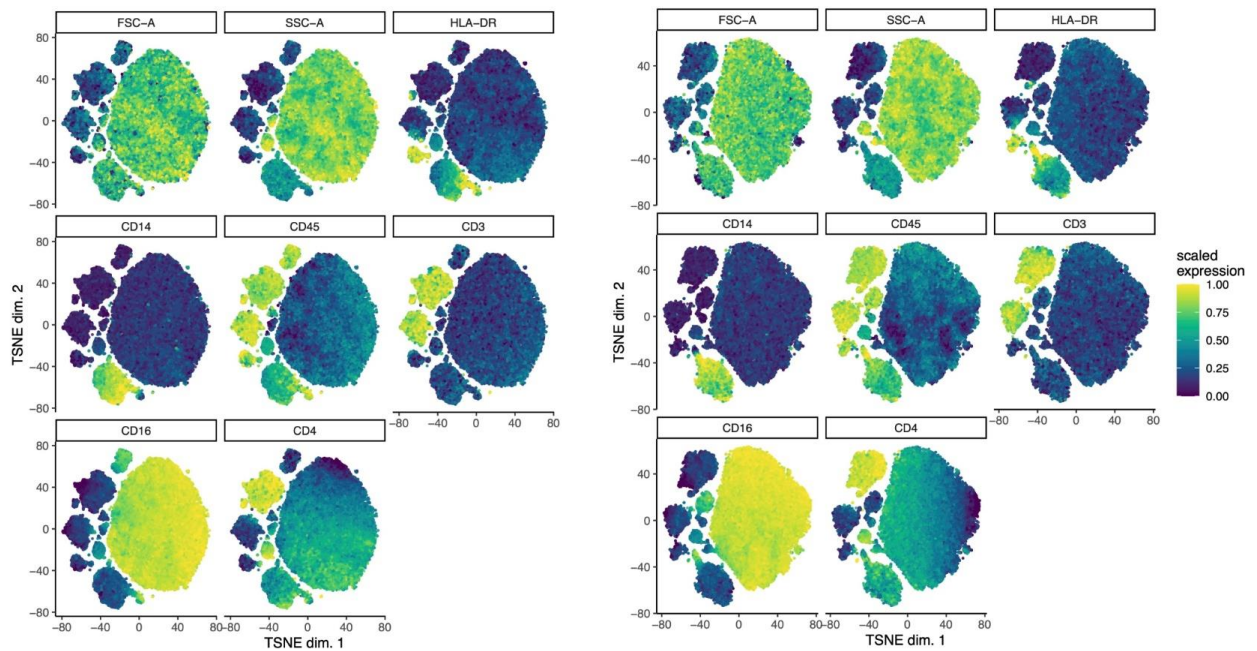
Supplementary Figure 1.



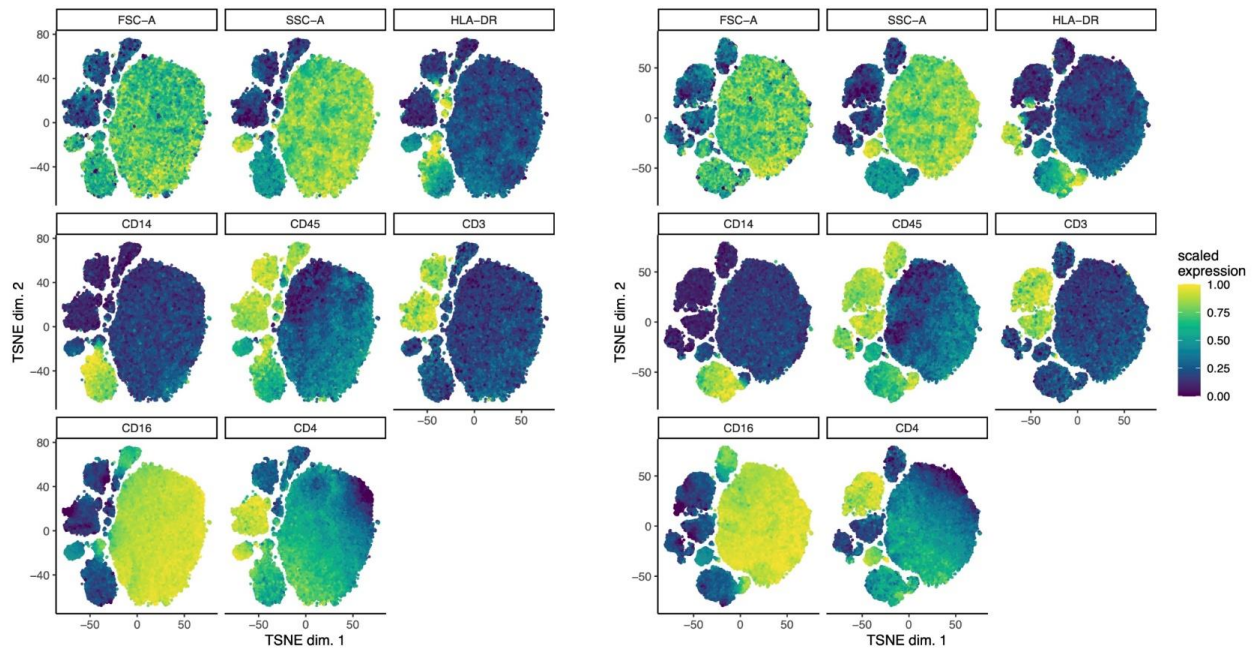
Supplementary Figure 1. Overall survival and immunological parameters evaluation in ICI-treated NSCLC patients. (A) Kaplan–Meier curves reporting the overall survival (OS, calculated from the date of start of ICI-treatment) of NSCLC patients ($n=34$) stratified by the clinical response to ICI treatment as non-progressor (NP, green) and progressor (P, red). (B) Representative gating strategy of flow cytometry analysis used for PMNs and lymphocyte subsets evaluation in fresh whole blood from NSCLC patients. (C) Neutrophil-to-lymphocyte (NLR), neutrophil-to-T lymphocyte (NTR), platelet-to lymphocyte (PLR), neutrophil-to-CD4⁺ lymphocyte, monocytes-to- lymphocyte (MLR), and monocytes-to- lymphocyte (MTR) ratios calculated by using circulating cell count in non-progressor (NP, green) and progressor (P, red) NSCLC patients before (T0) and after (T1) ICI treatment ($n=34$). Mann-Whitney and Wilcoxon test. Data are shown as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$. Stars and lines related to statistical analyses are indicated in: red for comparison between T0 and T1 in Progressor (P) patients; green for comparison between T0 and T1 in Non-Progressor (NP) patients; black for comparison between P and NP patients at either T0 (left) or T1 (right), respectively.

Supplementary Figure 2.

A

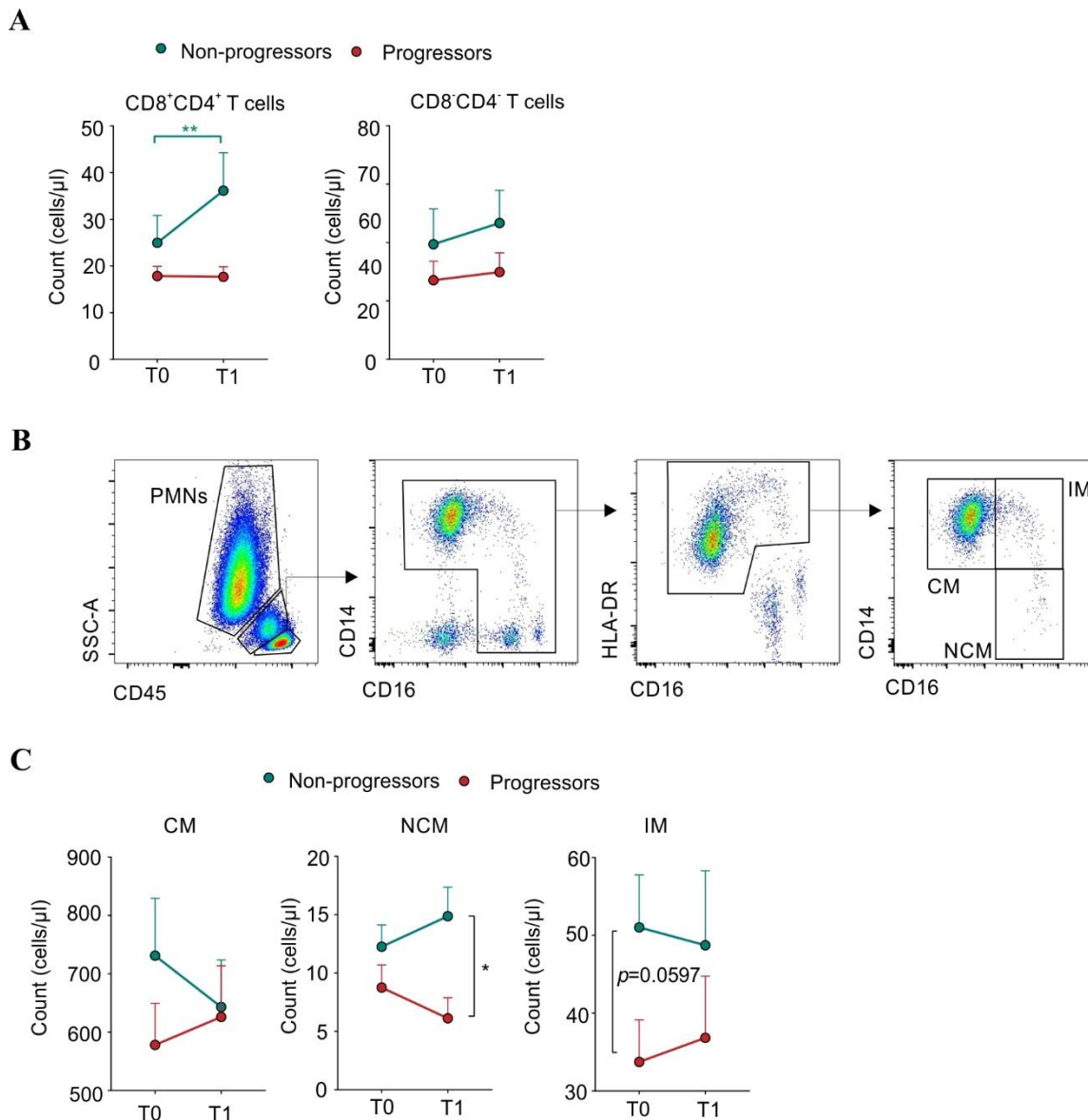


B



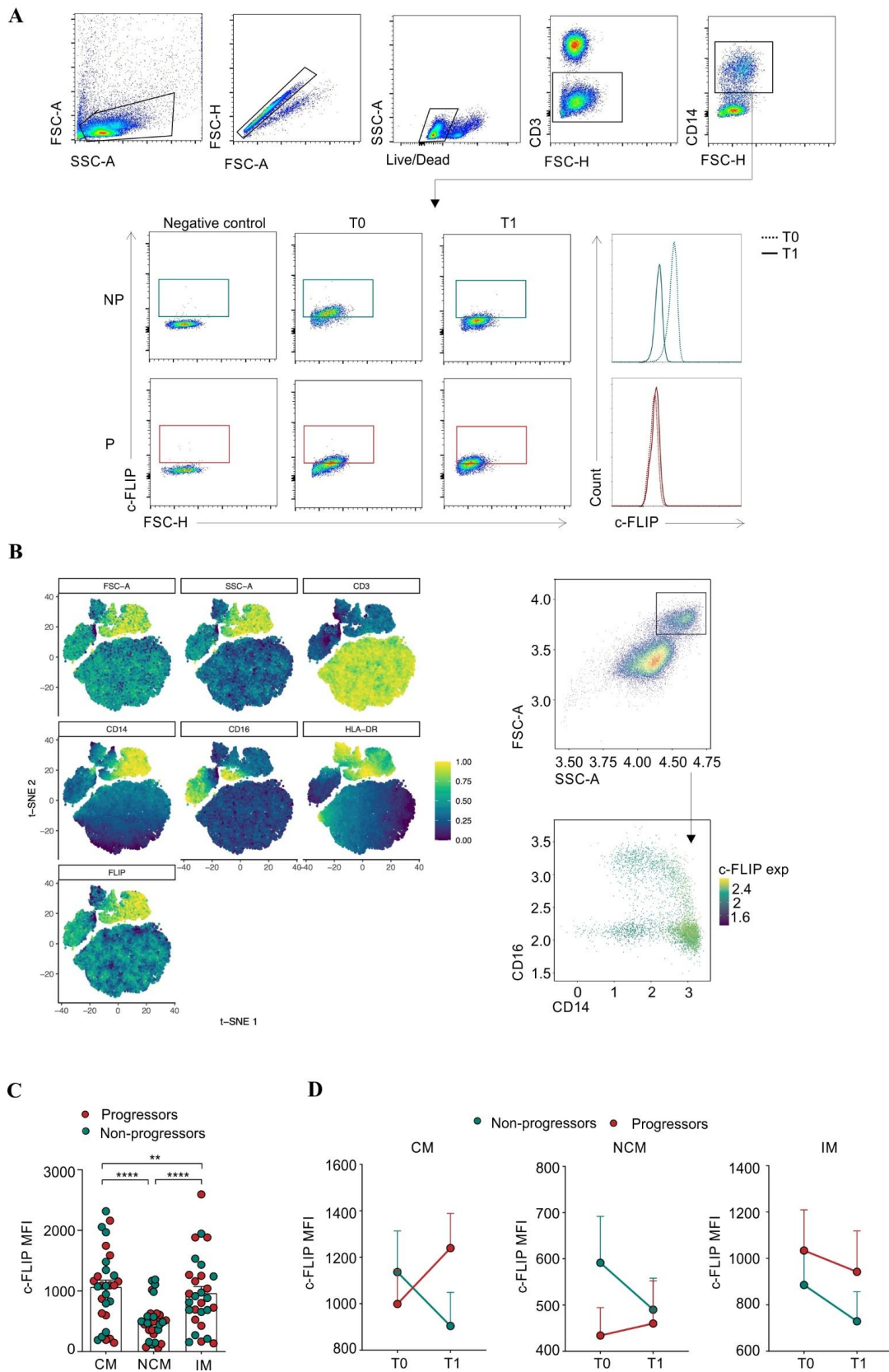
Supplementary Figure 2. Marker's expression on different subsets in t-SNE representation in NSCLC patients before and after ICI immunotherapy. t-SNE representation with the scaled expression of single markers used to annotate the cell clusters in (A) non-progressor patients at T0 (left) and T1 (right) and (B) progressor patients at T0 (left) and T1 (right).

Supplementary Figure 3.



Supplementary Figure 3. Evaluation of specific T lymphocyte and monocyte subsets in NSCLC patients before and after ICI immunotherapy. (A) Circulating CD8⁺CD4⁺ and CD8⁻CD4⁺ T cell count in the whole blood from non-progressor (NP, green line) and progressor (P, red line) NSCLC patients before (T0) and after (T1) ICI treatment (T0-P, n=16; T1-P, n=9, T0-NP, n=15, T1-NP, n=15). (B) Representative gating strategy of flow cytometry analysis used for monocyte subsets evaluation in fresh whole blood from NSCLC patients. (C) Circulating CM, NCM, and IM cell count in whole blood from non-progressor (NP, green) and progressor (P, red) NSCLC patients before (T0) and after (T1) ICI treatment (T0-P n=16, T1-P n=9, T0-NP n=15, T1-NP n=15). Mann-Whitney and Wilcoxon test. Data are shown as the mean \pm SEM. * $p < 0.05$, ** $p < 0.01$. CM: classical monocytes, NCM: non-classical monocytes, IM: intermediate monocytes.

Supplementary Figure 4.



Supplementary Figure 4. c-FLIP expression in M-MDSCs and monocyte subset in NSCLC patients before and after ICI immunotherapy. (A) Representative gating strategy of flow

cytometry analysis used for circulating c-FLIP expressing CD14⁺ cell evaluation in PBMCs isolated from NSCLC patients. **(B)** *t*-SNE representation with the scaled expression of single markers including c-FLIP (left). Gating strategy to extrapolate monocytic cells to assess c-FLIP expression by using the functions 'rectangleGate' and 'filter' of the 'flowCore' package (left). **(C)** c-FLIP expression evaluated by FACS analysis and reported as mean fluorescence intensity (MFI), FMO (fluorescence minus one) corrected, in circulating CM, NCM, and IM of non-progressor (NP, green) and progressor (P, red) NSCLC patients before (T0) ICI treatment. **(D)** c-FLIP expression evaluated by FACS analysis and reported as mean fluorescence intensity (MFI), FMO (fluorescence minus one) corrected, in circulating CM, NCM, and IM of non-progressor (NP, green) and progressor (P, red) NSCLC patients before (T0) and after (T1) ICI treatment. Mann-Whitney and Wilcoxon test. Data are shown as the mean \pm SEM. **** $p < 0.0001$. CM: classical monocytes, NCM: non-classical monocytes, IM: intermediate monocytes.