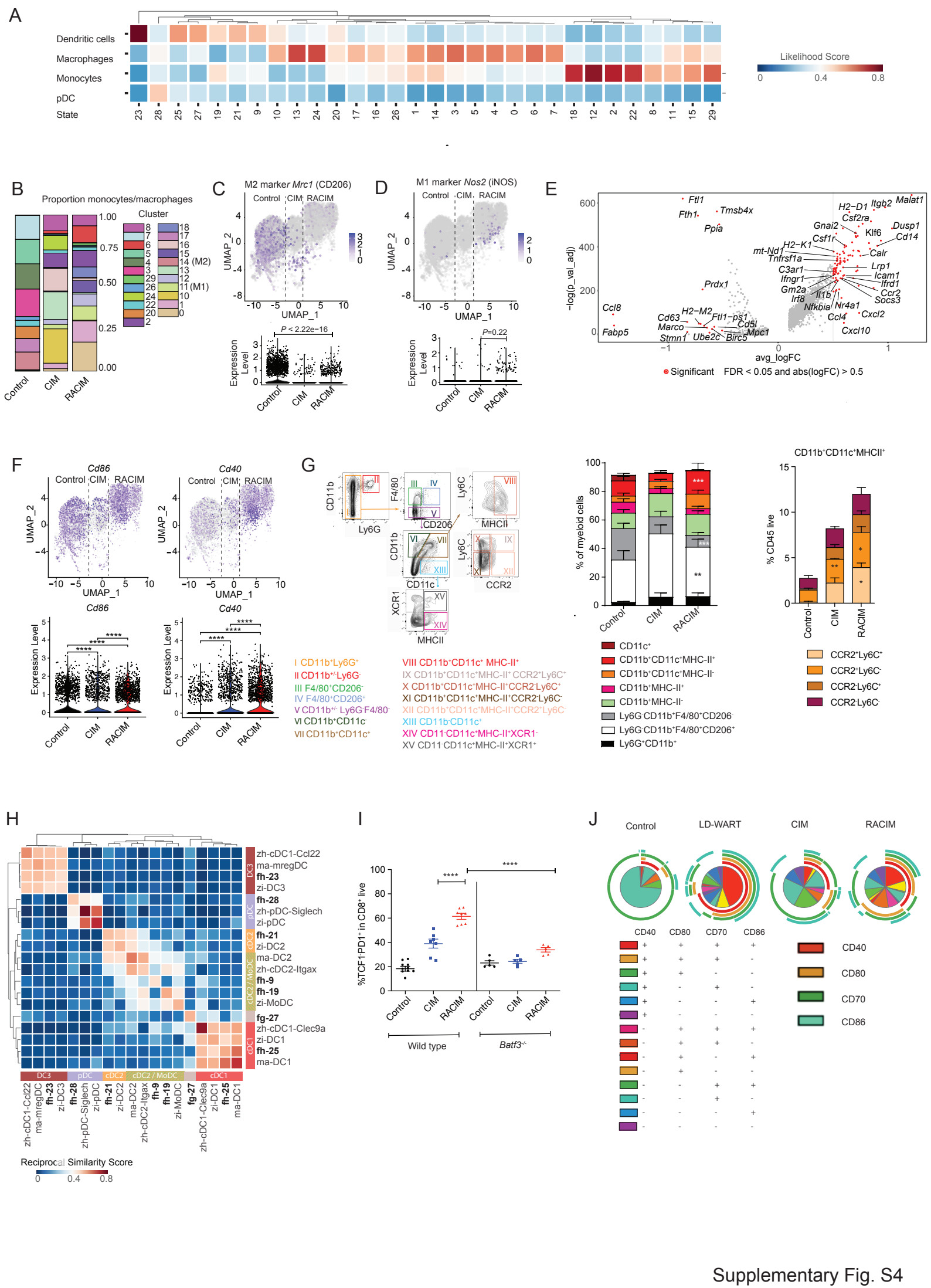

Supplementary Fig. S4

Supplementary Fig S4. Effect of therapy on the phenotype of intratumoral dendritic cells and macrophages. (A) Heatmap of scRNAseq data depicting the likelihood score of each myeloid cluster being a dendritic cell (DC), pDC, monocyte, or macrophage cell-type. Likelihood scores were returned by a linear support vector classifier fitted to log2 transformed single-cell transcriptome data from Zillionis et al. (35) **(B)** Stacked bar plot showing the proportion of the different monocytes/macrophage clusters in the three treatment conditions (CIM, RACIM or control) by scRNAseq. **(C-D)** UMAP projection of macrophages shows **(C)** *Mrc1* (CD206) and **(D)** *Nos2* (iNOS) single-cell transcripts clustered in the high-dimensional space in the three treatment conditions and quantified using violin plot. **(E)** Volcano plot showing changes in gene expression in the macrophage compartment of RACIM *vs.* CIM treated tumors. Each dot represents one gene, the genes are red colored if they pass the thresholds for FDR and Log Fold Change; on the right: upregulated, and on the left: downregulated genes. The top genes by *P* value and Log Fold Change are labelled. **(F)** *Cd86* and *Cd40* transcript levels in macrophages represented in the UMAP projection and below as violin plots in the three treatment conditions. **(G)** Flow cytometry gating strategy (left) and bar plot representing percentages of myeloid cell populations as determined by flow cytometry (right). Samples were first gated in hematopoietic $CD45^+Aqua^-$ live cells. Gates were based on fluorescence minus one (FMO) control. Macrophages, i.e. $CD11b^+F4/80^+$ cells after exclusion of neutrophils ($CD11b^+Ly6G^{hi}$), were classified as M2 ($CD206^{hi}$) or M1 ($CD206^{neg}$). $CD11b^-CD11c^+XCR1^{hi}$ defined cDC1. $CD11b^+CD11c^+MHCII^+$ defined a mixture of conventional (c)DC (i.e. cDC2 or cDC3) and $Ly6C^+$ monocyte-derived DC expressing CCR2. **(H)** Heat-map showing a reciprocal similarity score *r* for each tumor-infiltrating DC state comparison pair. The score was calculated using the probability estimates returned by the Linear Support Vector classifier on log2 transformed data. Each cell state name has a study prefix. Specific references are as follows: this study (fh), Zilionis et al., (zi; 35); Maier et al., (ma; 38)[32]; Zhang et al., (zh; 39)[33]. Five conserved tumor-infiltrating DC states were identified as follows: cDC1 (red), cDC2 (orange), cDC2/MoDC (orange-green striped), DC3 (dark red) and pDC (violet). **(I)** % intratumoral $TCF1^-PD1^+$ $CD8^+$ cells in wild-type *vs.* *Batf3*$^{-/-}$ tumor bearing mice by flow cytometry after indicated treatments. **(J)** SPICE plots summarizing FACS data for the co-expression of CD40, CD80, CD70 and CD86 on intratumoral $CD11b^+CD11c^+MHCII^+$ cells isolated from the different treatment conditions as indicated. Flow cytometry data are representative of 3 independent experiments (n=5-10 mice/group). Statistical analysis was performed using Student's unpaired *t*-test, error bars represent mean ± SD. * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.