Cell Reports, Volume *39*

Supplemental information

Metabolism drives macrophage heterogeneity in the tumor microenvironment

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Supplemental Information
Figures S1-S6

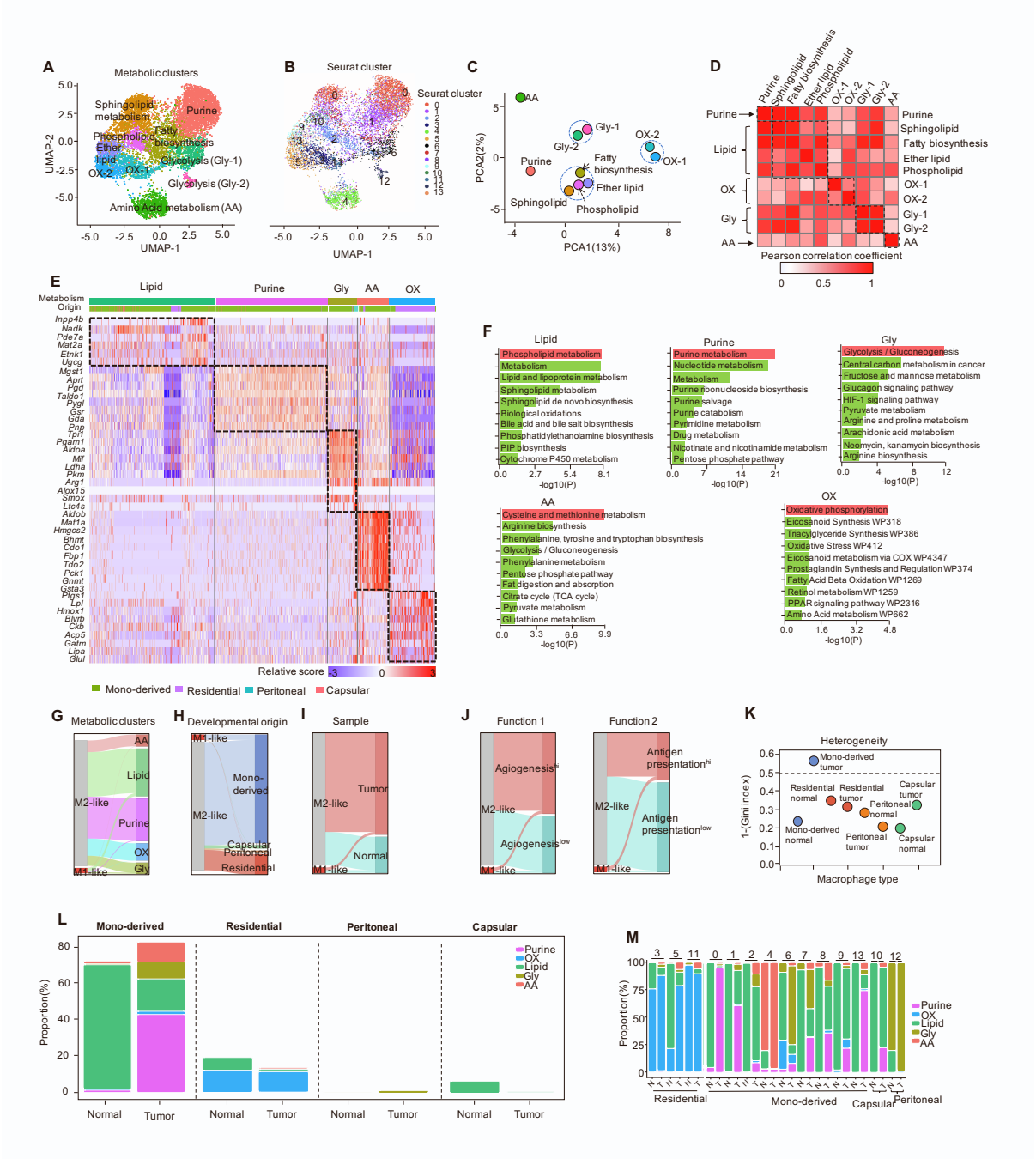

Figure S1. TAMs manifest high metabolic diversification. (A-B). UMAP plots showing the metabolic clusters of macrophages. The color of each dot in each panel respectively indicates: the 10 dominant metabolic clusters determined with 1310 metabolic genes **(A)**, and the 14 original Seurat clusters determined with the macrophage markers as showed in Figure 1B **(B)**, Gly: glycolysis, OX: OXPHOS. **(C).** Principal component analysis (PCA) for 10 metabolic clusters with all variable genes in each cluster, averaged position of all cells in each cluster were represented, dots were colored with the same color as showed in **(A)**, Gly: glycolysis, OX: OXPHOS, AA, Amino acid metabolism, Purine: Purine metabolism. **(D).** Pearson correlation coefficient matrix of 10 metabolic clusters, Gly: glycolysis, OX: OXPHOS, AA: Amino acid metabolism, Purine: Purine metabolism, Lipid: Lipid metabolism. **(E).** Heatmap showing the expression level of top 8 (if available) significant enriched metabolic genes (only metabolic genes were considered) in 5 metabolic clusters of macrophages. 10 metabolic clusters showed in **(A)** were merged into 5 clusters: four lipid related metabolic clusters of fatty biosynthesis, phospholipid, ether lipid, and phospholipid were merged as "Lipid", two oxidative phosphorylation clusters were merged as "OX", two glycolysis clusters were merged as "Gly" (as showed in Figure 2E), AA: Amino acid metabolism, Purine: Purine metabolism. **(F).** Gene Ontology analysis for top 8 (if available) significant enriched metabolic marker genes in 5 metabolic clusters of macrophages. **(G-J).** Sankey diagram showing the distribution of macrophages in five metabolic clusters **(G),** in four

developmental origins **(H)**, in two samples they were isolated from **(I)**, and in two macrophage functions **(J)** respectively, classified by M1/M2-like macrophages. **(K).** Heterogeneity of macrophages from different origins in tumor and normal. Heterogeneity: 1- (Gini index), which was calculated by "ineq". Dash: sample with significant diversity. **(L).** Relative proportion of 5 metabolic cluster in macrophages from different origins versus samples from normal and tumor. **(M).** Relative proportion of 5 metabolic cluster in each population determined in Figure 2L versus samples from normal and tumor. Related to Figure 2.

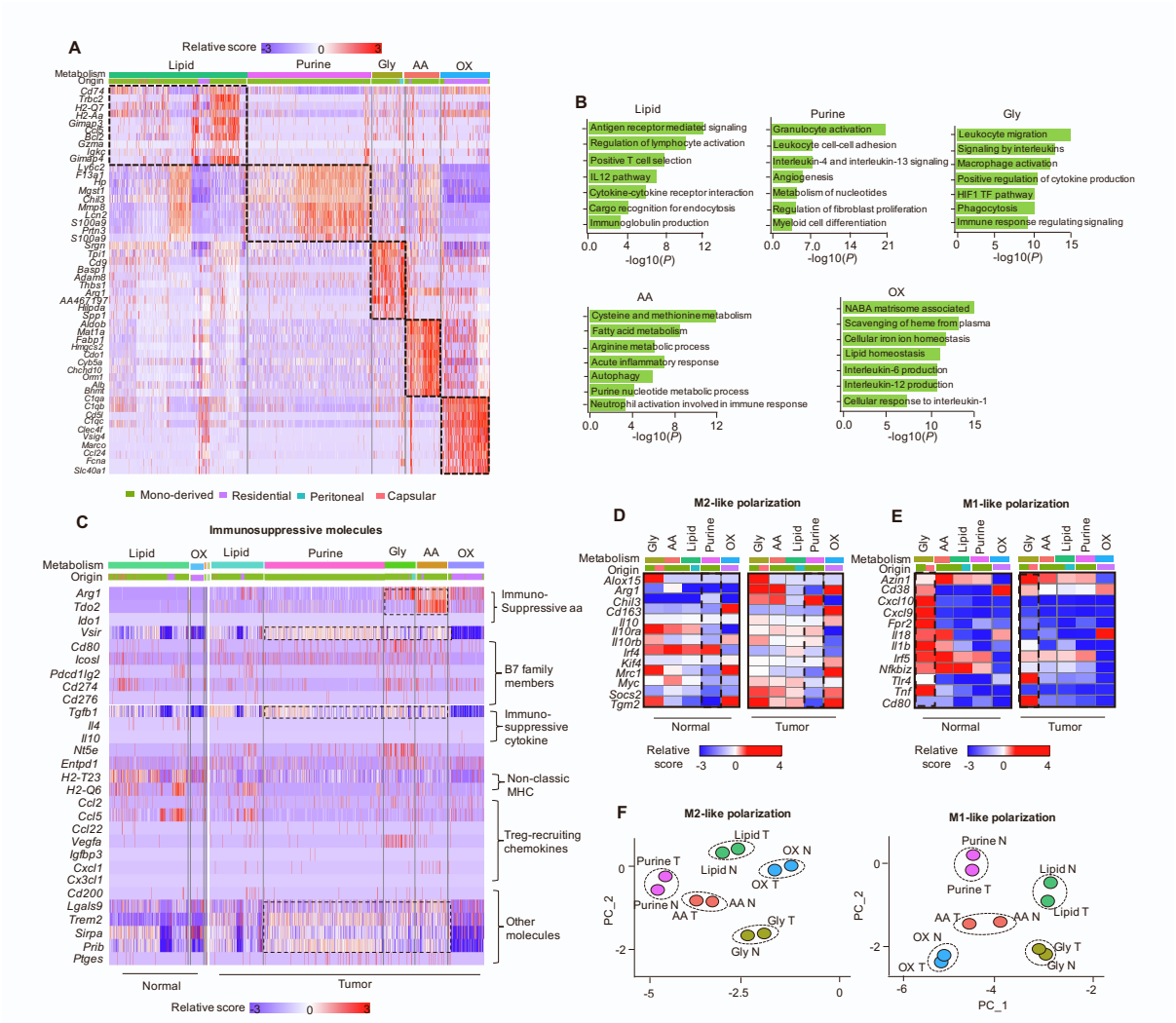

Figure S2. Metabolic profiles correlate to distinct functional programs in TAMs. (A). Heatmap showing the expression level of top 8 (if available) significant enriched marker genes (all genes were included) in metabolic clusters ofmacrophages. **(B).** Gene Ontology analysis for significant marker genes (min.pct = 0.25, logfc.threshold = 0.25) in each of the 5 metabolic clusters. (**C**). Heatmap showing the expression level of 29 immunosuppressive molecules with 6 different immunosuppressive mechanism of macrophages in five metabolic clusters. Each heatmap column represents one cell. **(D-E).** Heatmap showing the expression level of signature genes for M2-like macrophage (**D)** and M1-like macrophage (**E)** in five metabolic clusters. Color of each heatmap cell represents the relative expression level of each gene (Z-score), each heatmap column represents one cluster. **(F).** PCA of 5 metabolic clusters with the expression level of M2-like (left) and M1-like (right) gene signature. Cells from normal and tumor were analyzed separately, N: normal, T: tumor, Lipid: Lipid metabolism, Purine: Purine metabolism, Gly: glycolysis, AA: Amino acid metabolism, OX: OXPHOS. Related to Figure 3.

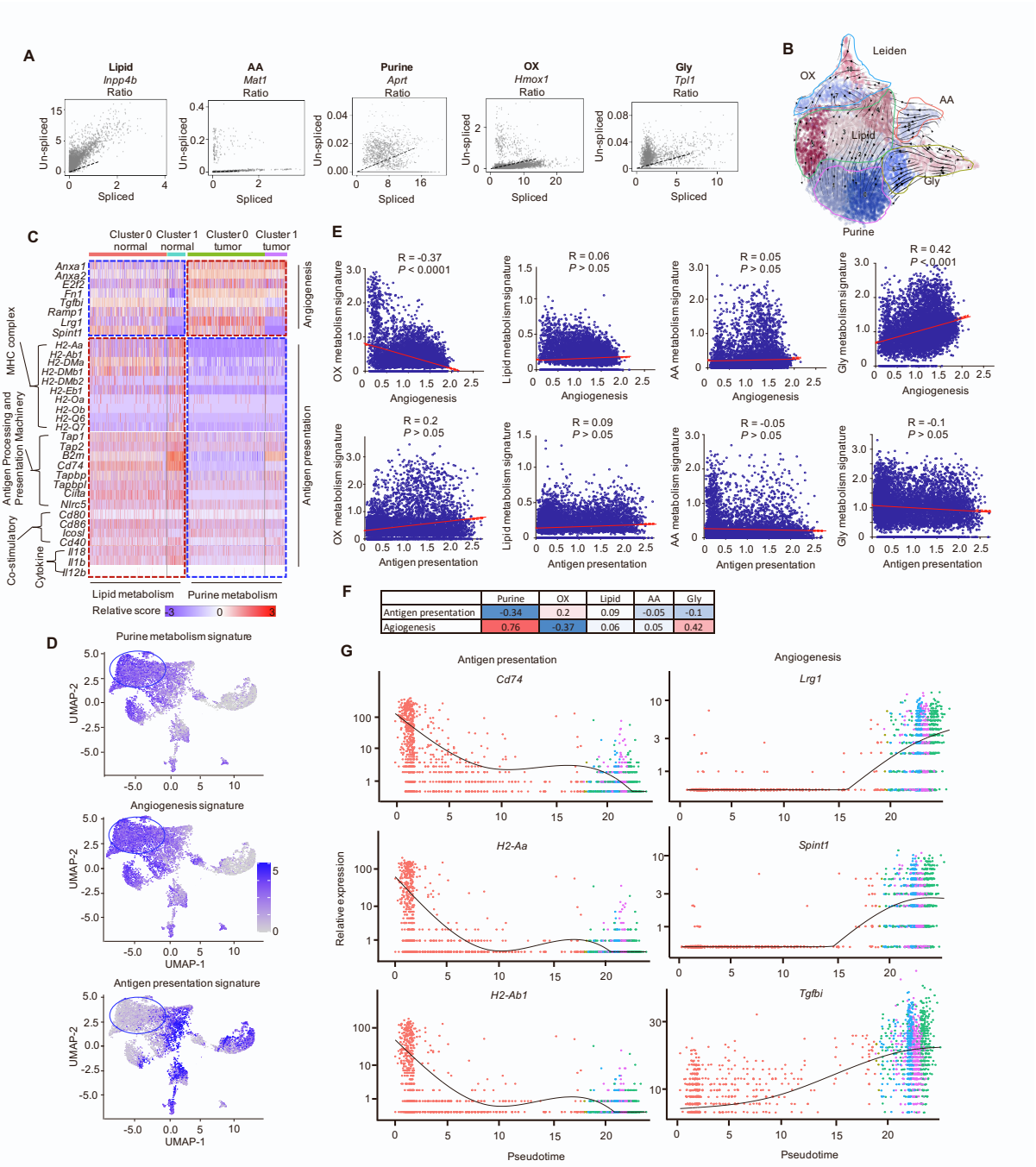

Figure S3. Purine metabolic TAMs display weak antigen presentation and potent angiogenesis potential
(A). The ratio of unsliced to spliced mRNA abundance of selected metabolic marker genes in each metabolic cluster on the Louvain projection modeled by scVelo. The black line: the estimated 'steady-state' ratio. RNA velocity is determined by how much an observation deviates from the steady-state line, higher abundance of unspliced mRNA than expected in steady state means positive velocity and up-regulated of a gene. **(B).** RNA velocity of 5 metabolic clusters overlaid with RNA velocity stream. Cells were colored by Leiden cluster generated by scVelo. **(C)**. Heatmap showing the expression level of enriched and deleted genes of purine metabolism dominant macrophages compared to lipid metabolism dominant macrophages. Macrophages from cluster 0 and cluster 1 (as showed in Figure 2L) in both normal and tumor samples were selected. **(D)**. Enrichment of gene signatures of purine metabolism, angiogenesis and antigen presentation in each macrophage. **(E)**. Correlation analysis between gene signatures of OX, lipid, AA as well as glycolysis (y-axis) and functional programs of angiogenesis and antigen presentation (x-axis) on macrophages. Pearson's correlation coefficient (R) and correlation test (P) were used to evaluate the association between two signatures. **(F)**. Summary of Pearson correlation coefficient between 5 metabolic gene signatures with 2 functional programs. Pearson's correlation coefficient (R) was used to evaluate the association between two signatures. **(G)**. The patterns of gene expression along with the pseudo-time. Related to Figure 4.

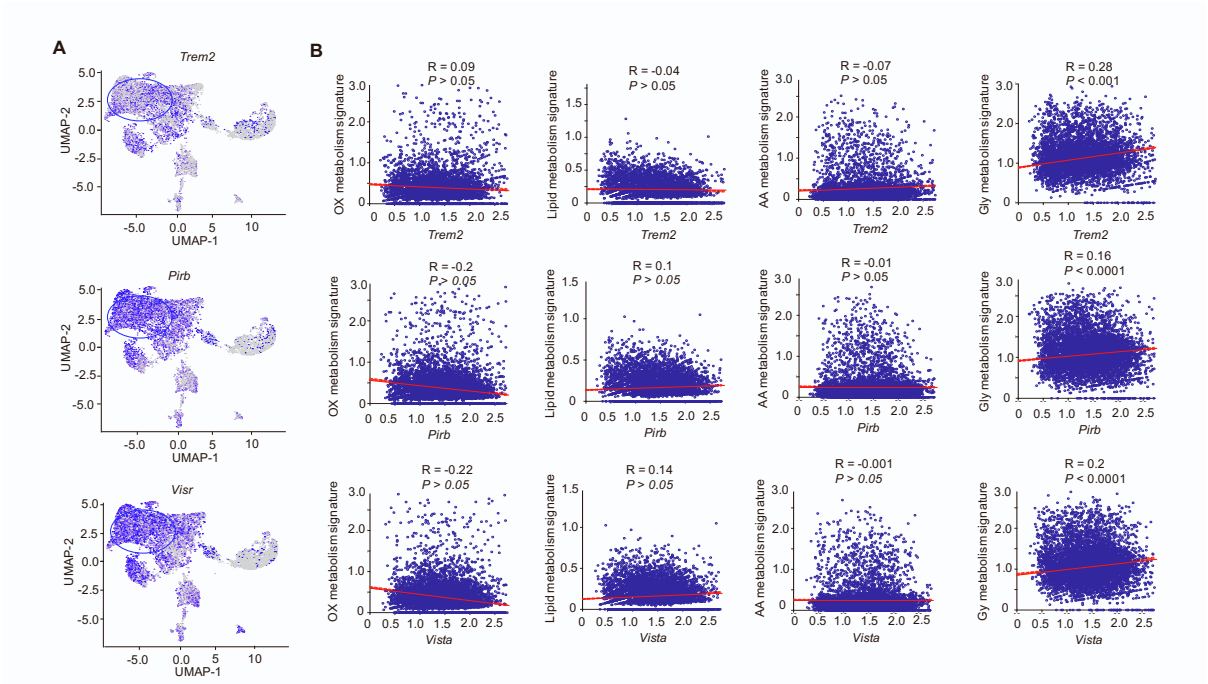

Figure S4. Purine metabolic TAMs express high immunosuppressive molecules (A). Enrichment of *Trem2*, *Pirb* and *Visr* in each macrophage. **(B)**. Correlation analysis between 4 metabolic gene signatures (y-axis) with 3 immunosuppressive molecules (x-axis) in macrophages. Pearson's correlation coefficient (R) and correlation test (P) were used to evaluate the correlation between individual metabolic signatures and individual immunosuppressive genes. Related to Figure 4.

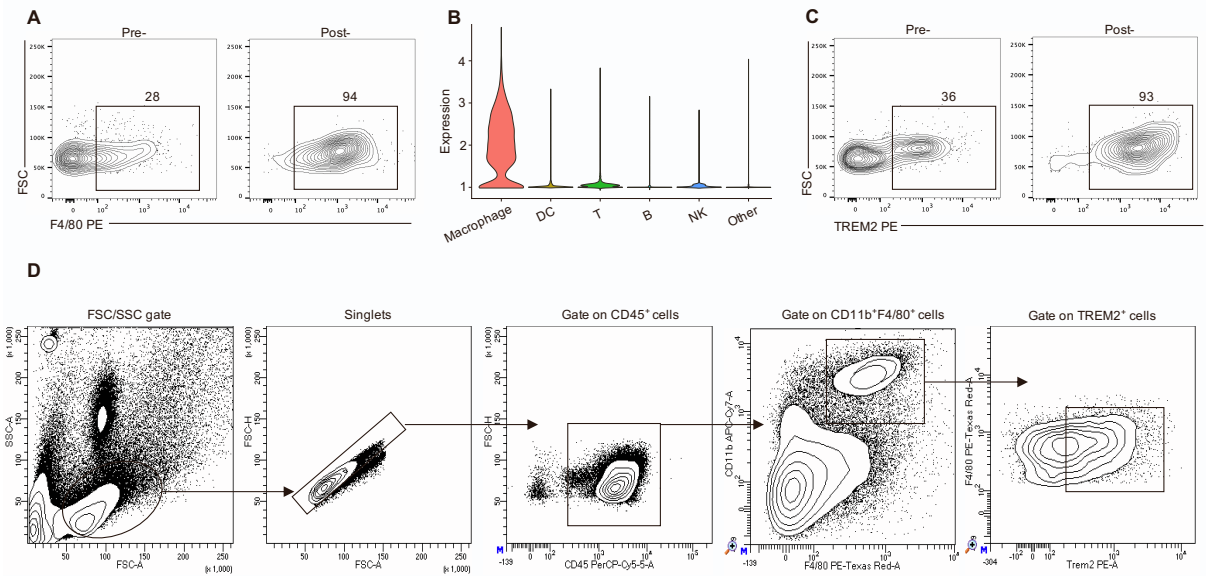

Figure S5. Sorting and FACS gating TREM2$^+$ macrophages (A). Purity of F4/80$^+$ cells post sorting. **(B).** Expression of *Trem2* on *Cd45*$^+$ cells. **(C).** Purity of TREM2$^+$ cells post sorting. **(D).** Gating strategy of TREM2$^+$ macrophages. Related to Figure 5.

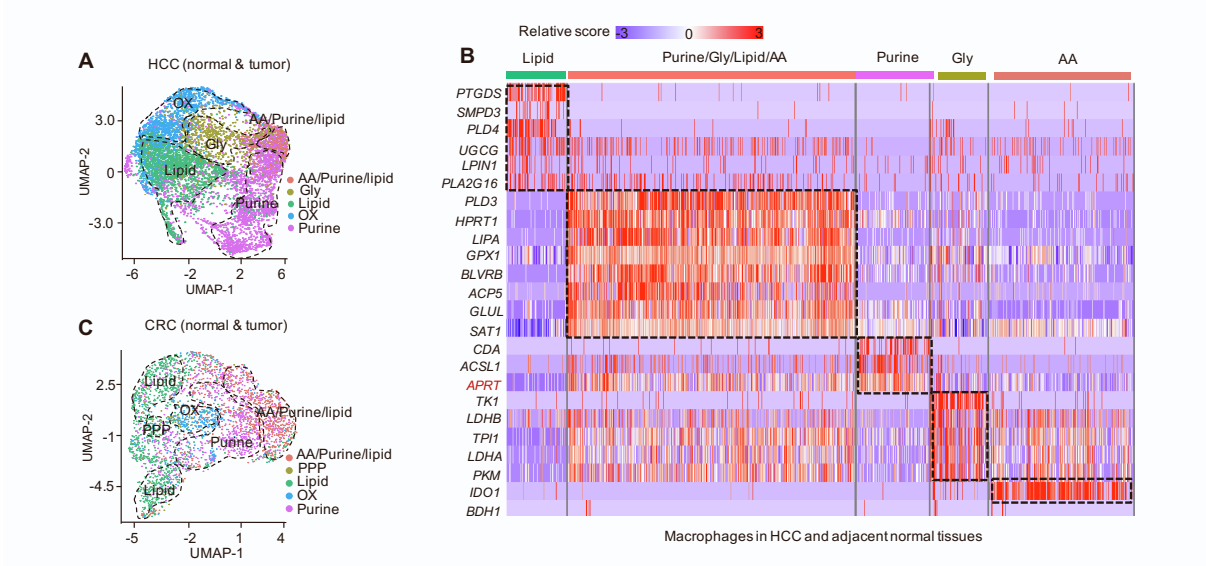

Figure S6. Human TAMs exhibit high metabolic heterogeneity (A). UMAP plots showing the metabolic clusters of macrophages from human tumor cohort of HCC. Metabolic clusters were determined with the same method with dataset from mice, as showed in Figure 2D, with the same 1310 metabolic genes. **(B).** Heatmap showing the expression level of top significant enriched metabolic marker genes (only metabolic genes were considered) in metabolic clusters of 5353 macrophages from 14 HCC patients (Sharma et al., 2020). **(C).** UMAP plots showing the metabolic clusters of macrophages from human tumor cohort of CRC. Related to Figure 6.