

Supplementary Figure S1. (A) Hierarchical clustering of all differentially expressed genes (DEGs) between LUAD PBMC (n=5) and HD PBMC (n=4). Expression values are Z score transformed. Samples were clustered using complete linkage and Euclidean distance. (B) Volcano plot showing upregulated (red dots) and downregulated genes (green dots) resulted from the differential expression analysis performed on LUAD PBMC vs HD PBMC. Only genes with $|\text{Log}_2\text{FC}| > 2$ and $\text{adjp_val} < 0.05$ were considered significantly deregulated. Some of the most relevant genes are reported. (C) Gene Ontology (GO) analysis of downregulated genes (upper panel) and upregulated genes (lower panel) in LUAD PBMC vs HD PBMC. Terms over red dashed line are considered statistically significant ($\text{adjp_val} < 0.05$). (D) qRT-PCR validation showing mRNA levels in LUAD PBMC (blue) compared to HD PBMC (red) of some selected proinflammatory cytokines and chemokines genes (GAPDH was used as housekeeping gene). The box plot represents minimum and maximum values (whiskers), median values (center lines), mean values (center asterisk) and 25th and 75th percentiles (box edges), with all data points plotted. Each dot represents one patient (healthy: n= 8; patients: n=14). * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$ (Wilcoxon Signed Rank Sum Test). LUAD, lung adenocarcinoma; HD, healthy donors; PBMC, peripheral blood mononuclear cells; FC, fold change; adjp_val ; adjusted p_value.

Supplementary Figure S2. (A) Bar plots of selected deregulated genes, from RNA-Seq, in LUAD PEMC vs PBMC ($|\text{Log}_2\text{FC}| > 2$ and $\text{adjp_val} < 0.05$). The selected genes were grouped into three main clusters: growth factors and metastatic angiogenic promoters (left panel), proinflammatory chemokines (middle panel) and cytokines/enzymes related to the regulation of the immune response (right panel). (B) qRT-PCR analyses confirming the upregulation of proinflammatory cytokines and chemokines genes in LUAD PEMC (yellow) vs matched PBMC (blue). GAPDH was used as housekeeping gene. The box plot represents minimum and maximum values (whiskers), median values (center lines), mean values (center asterisk) and 25th and 75th percentiles (box edges), with all data points plotted. Each dot represents one patient (n=14). * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.001$ (Wilcoxon Signed Rank Sum Test). LUAD, lung adenocarcinoma; PEMC, pleural effusion mononuclear cells; PBMC, peripheral blood mononuclear cells; FC, fold change; adjp_val ; adjusted p_value.

Supplementary Figure S3. Dot plots showing the differential deconvolution analysis performed on lymphoid and myeloid subsets between the three sample groups. Each dot represents one patient. Mean values of CIBERSORTx absolute scores and standard deviations for each cell subset were calculated for each patient group and compared using paired (LUAD PEMC vs PBMC) or unpaired (LUAD PBMC vs HD) two-tailed Student's t-test. * $p < 0.05$; ** $p < 0.01$. LUAD, lung adenocarcinoma; HD, healthy donors; PBMC, peripheral blood mononuclear cells; PEMC, pleural effusion mononuclear cells.

Supplementary Figure S4. LUAD and HD plasma are characterized by similar cytokine pattern. Scatter plots showing the mean values \pm SEM of cytokines concentration (pg/ml), evaluated in plasma of 15 LUAD patients and 4 HD. LUAD, lung adenocarcinoma; HD, healthy donors.

Supplementary Figure S5. Representative images of wound healing assay of two MPE-derived primary cell lines, BBIRE-T248 (A) and PUC 36 (B), stimulated with 10% of their matched cell free MPEs and respective non-treated cells (CTRL), immediately after insert removal (T0) and after 24 hours (T24) of treatment. Magnification: 4X. (C) Percentage of open residual wound area after 24 hours (T24) respect to T0 in BBIRE-T248 and PUC 36 (D) treated cells (MPEs) and in the respective non-treated cells. The mean \pm SEM of three independent experiments is reported. * $p < 0.05$; and **** $p < 0.0001$ (paired Student's t-test).

Supplementary Figure S6. RNA expression levels of MDM (A) and TRM (B) signatures are tested across different immune cell types. Boxplots show the mean-normalized expression value of each gene in each selected cell type. The analysis was performed through My GenSet online tool. Data from <http://www.immgen.org>. (C) Kaplan Meier analysis of overall survival comparing LUAD patients with high (red curves) and low (black curves) expression of MDM and TRM (D) signatures. $p < 0.05$ was considered as statistically significant (log rank test). Data Plotted from <http://kmpplot.com>. LUAD, lung adenocarcinoma; TRM, tissue resident macrophages; MDM, monocytes derived macrophages.

Supplementary Figure S7. Box plots representing the mean expression values of the MPEs Mφ signature across different LUAD stages. Stage I (patients: n=274), Stage II (patients: n=122), Stage III (patients: n=83), Stage IV (patients: n=26).