**Regulon analysis**

Regulon (gene-regulatory network) analysis was performed using pySCENIC (version 0.12.0) to derive a set of regulons likely driving the differentiation from SPP1+MAM-to SPP1+MAM+ macrophages [105]. Briefly, pySCENIC (1) derives a set of gene co-expression network defined by a transcription factor (TF) and its target genes, (2) evaluates a network for enrichment of TF-specific cis-regulatory element and removes targets genes lacking an enrichment of these elements, and (3) assesses the activity level of the network in each individual cell by an “Area Under the Curve Score” (AUC). We ran pySCENIC with default parameterization on SPP1+MAM-and SPP1+MAM+ macrophages. The expression data from integrated assay (output of Seurat’s integration pipeline) (24,105 cells by 3,000 genes) were used as input, and a list of human-specific TF was downloaded from [github.com/aertslab/pySCENIC/blob/master/resources/hs\_hgnc\_tfs.txt](https://github.com/aertslab/pySCENIC/blob/master/resources/hs_hgnc_tfs.txt). For each gene in the transcriptome, a tree-based regression model was built with the TF candidates as predictors using GRNBoost2. In step 2 (network refinement), we used the following database of genome-wide regulatory features ([hg19-500bp-upstream-10species.mc9nr.genes\_vs\_motifs.rankings.feather](https://resources.aertslab.org/cistarget/databases/homo_sapiens/hg19/refseq_r45/mc9nr/gene_based/hg19-500bp-upstream-10species.mc9nr.genes_vs_motifs.rankings.feather), [hg19-tss-centered-5kb-10species.mc9nr.genes\_vs\_motifs.rankings.feather](https://resources.aertslab.org/cistarget/databases/homo_sapiens/hg19/refseq_r45/mc9nr/gene_based/hg19-tss-centered-5kb-10species.mc9nr.genes_vs_motifs.rankings.feather)) and TF motifs ([motifs-v9-nr.hgnc-m0.001-o0.0.tbl](https://resources.aertslab.org/cistarget/motif2tf/motifs-v9-nr.hgnc-m0.001-o0.0.tbl)) provided by laboratory of Serin Aerts to assess a regulon for the enrichment of regulatory features and prune the member genes. In brief, these database files contain pre-computed rankings of genome-wise regulatory features in the target genes. In step 3 (the evaluation of regulon activity), pySCNEIC ranked each gene in the transcriptome of a cell by its expression, and an AUC score evaluates the enrichment of the members in a regulon based on this ranking. The activation status of a regulon in a cell is finally derived by binarizing the AUC score.

In total, 238 regulons were identified, and we only retained regulons which are activated in at least 10% of the cells in at least 4 tissues, resulting in 173 regulons. We evaluated the specificity of a regulon to SPP1+MAM+ macrophages using three criteria: (1) the specificity to the activation status of the regulon with respect to SPP1+MAM+ macrophages (relative to SPP1+MAM- macrophages), (2) the specificity of the regulon to the core signature of SPP1+MAM+ (see **SPP1+MAM+ transcriptomic signature** for details) and (3) the potential of the regulon to promote the polarization of SPP1+MAM- macrophages towards the SPP1+MAM+ state.

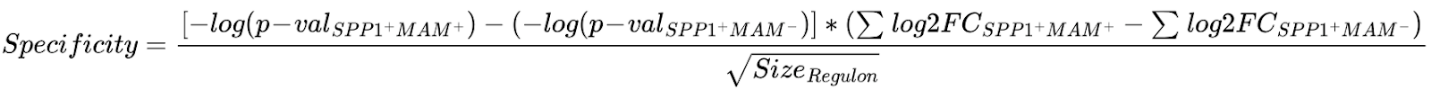
*First criterion* - we evaluated the specificity of the activation of a regulon in SPP1+MAM+ using the Diagnostic Odd Ratio (DOR) [106]. Briefly, DOR assesses the odds of a positive test in “cases” relative to the odds of a positive test in “controls”. Here we refer to the activation of the regulon in SPP1+MAM+ as “cases” and the activation of the regulon in SPP1+MAM- macrophage as “control”. The DOR for a regulon is calculated using the following formula:



where TP refers to number of SPP1+MAM+ in which the regulon is activated, TN the number of SPP1+MAM- macrophages in which the regulon is not activated, FP the number of SPP1+MAM- macrophages in which the regulon is activated, and FN the number of SPP1+MAM+ macrophages in which the regulon is not activated.

DOR of each regulon is calculated for each tissue and median DOR across 6 tissues is taken as final score of regulon activation in SPP1+MAM+ macrophages.

*Second criterion* - to assess the specificity of a regulon to the core signature of SPP1+MAM+ macrophages, we examined (1) the size of overlap between the regulon and the signature and (2) the importance of the genes in the overlap. Specifically, we first performed a hypergeometric test to evaluate the significance of the overlap between the core SPP1+MAM+ signature and member genes of the regulon. We then calculated and summed over the gene expression fold changes between the SPP1+MAM+ to SPP1+MAM- macrophage states of the overlapping genes. We repeated the same calculation (both hypergeometric test and fold change calculation) for genes downregulated in SPP1+MAM+ compared to SPP1+MAM- (log2FC<-0.5) The specificity of a regulon to core SPP1+MAM+ signature was then calculated using the following formula:



where “p-val” refers to the p-value of the hypergeometric test, and SizeRegulon is the number of genes in the regulon.

The expression of a regulon is calculated using AddModuleScore() function in *Seurat* R package.

*Third criterion* - to delineate which regulon is more specifically required for the polarization of SPP1+MAM- macrophages to the SPP1+MAM+ state (as opposed to the possible differentiation from transitional to SPP1+MAM-macrophages), we pooled together SPP1+MAM+, transitional and SPP1+MAM- macrophages from all tissue and plotted the activity score of a regulon in each macrophage against the activity score of SPP1+MAM+ signature (**Figure 4C**) for selected regulons (see **Supplementary Table 9** for member genes of each regulon in this analysis). Activity score AUC is calculated using *AUCell* R package, which is the same as the final step of pySCENIC. Here, we evaluated the potential of SPP1+MAM+ macrophages to acquire the SPP1+MAM+ polarization state using the expression of SPP1+MAM+ signature. We performed linear regression analyses of regulon expression against expression of the SPP1+MAM+ signature, using the transitional and SPP1+MAM- macrophages, or using SPP1+MAM- and SPP1+MAM+ macrophages. We classified the regulon with greater increase in regulon expression within the SPP1+MAM- and SPP1+MAM+ macrophages (as compared to within SPP1+MAM- and transitional macrophages) as the regulon most likely to drive the differentiation progression from SPP1+MAM- macrophages to the SPP1+MAM+ polarization state.