Figure 4. A core set of regulons drives the polarization of SPP1+ MAM- to SPP1+ MAM+

**(A)** Rank plot for the 173 regulons active in SPP1+ MAM+ macrophages and driving the differentiation from SPP1+ MAM- to SPP1+ MAM+ macrophages. For each regulon, the diagnostic Odd Ratio (DOR) (y-axis) calculates the odds of being activated in a SPP1+ MAM+ over the odds of being activated in a SPP1+ MAM- macrophages (see Methods for details). A positive DOR is associated with the regulon activation in SPP1+ MAM+. DOR values are reported as log10(DOR). **(B)** Scatterplot plots the specificity of regulon genes to the transcriptomic signature of SPP1+ MAM+ (y-axis) against the difference in expression of a regulon between SPP1+ MAM+ and SPP1+ MAM- macrophages (x-axis). Briefly, the specificity score of the regulon reflects the degree of overlap between regulon genes and the SPP1+ MAM+ signature genes (see Methods for details). **(C)** Scatterplot showing each cell’s activity score of selected regulons (y-axis) against the cell’s SPP1+ MAM+ transcriptomic signature activity score (x-axis). These scores are shown for all the transitional, SPP1+ MAM- and SPP1+ MAM+ macrophages pulled together across tissues, and each dot on the scatterplot is a macrophage coloured by its cell-type identity. For each regulon, separate linear regression models have been fitted to each trajectory: (i) from the transitional to SPP1+ MAM- macrophages (black regression line), and (ii) from SPP1+ MAM- to SPP1+ MAM+ macrophages (red regression line). We defined two patterns of change in the regulon activity score: pattern (1) where there is a change in the regression slopes between the two differentiation trajectories, and pattern (2) with no apparent change in the regression slopes (see illustrations on the right). For each regulon, the pattern is indicated in the scatterplot (insert), and the slope of the linear regression and the Spearman correlation (ρ) are indicated. **(D)** UMAP of SPP1+ macrophages coloured by expression level of selected regulons, and by whether the cell is SPP1+MAM+ or SPP1+MAM-. **(E)** Box plots summarizing the fold change of regulon gene expression from SPP1+ MAM+, SPP1+ MAM-, transitional macrophages compared with regulon expression in homeostatic macrophages. Within each tissue, the ratio (fold change, FC) between the regulon activity score (AUC) in each cell from SPP1+ MAM+, SPP1+ MAM-, or transitional macrophage populations with respect to the median AUC in the homeostatic macrophages was calculated. The differences in FCs in regulon expression between SPP1+ MAM+ macrophages and other macrophages (i.e., SPP1+ MAM+ vs SPP1+ MAM-, SPP1+ MAM+ vs transitional macrophages, or SPP1+ MAM+ vs homeostatic macrophages) were all statistically significant (adjusted p-value < 0.05) evaluated using a two-tailed Wilcoxon rank-sum test. Adjusted P-value for each comparisons between SPP1+MAM- macrophages and SPP1+ MAM+ macrophages were indicated.