

Supplementary Materials

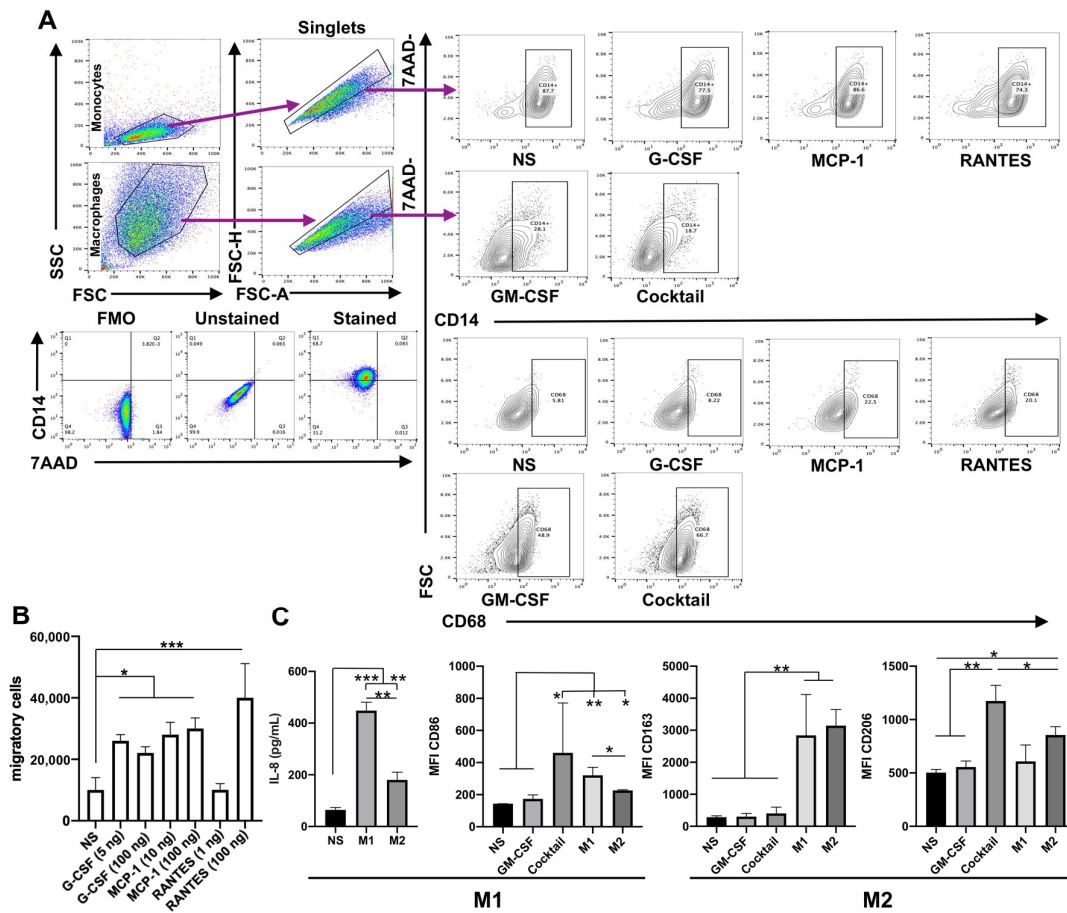


Figure S1. GM-CSF, G-CSF, MCP-1 and RANTES proinflammatory cytokines promote PBM differentiation into M1-like macrophages. **(A)** Flow cytometry of CD14 and CD68. The gates were adjusted according to two populations with different morphological characteristics (size and complexity). The first group is made up of monocytic cells derived from the following conditions: NS and treated with G-CSF, MCP-1 and RANTES individually, and the second group is made of the macrophage-like cells generated with GM-CSF and the cocktail of all cytokines. All the analyzes were performed from live cells (7AAD-). Representative images of one experiment. **(B)** Migration assay of U937 monocytes. We used as chemotactic agent the cytokines at the concentrations found in the cocktail, in addition, we used 100 ng/mL of each cytokine, which is the concentration more often used in other studies. For the statistical analysis all conditions were compared against NS. **(C)** Evaluation of a panel of M1 (IL-8 and CD86) and M2 (CD163 and CD206) macrophage markers. NS: non-stimulated monocytes kept in culture for as many days as the cytokine-induced monocytes. Macrophages induced with either GM-CSF/LPS/IFN γ (M1) or M-CSF/IL-4/IL-13 (M2) were included for comparison. Data represent the mean \pm SEM (standard error of the mean) from three independent experiments. Statistical tests were the non-parametric Kruskal-Wallis and Dunnett post-hoc tests, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

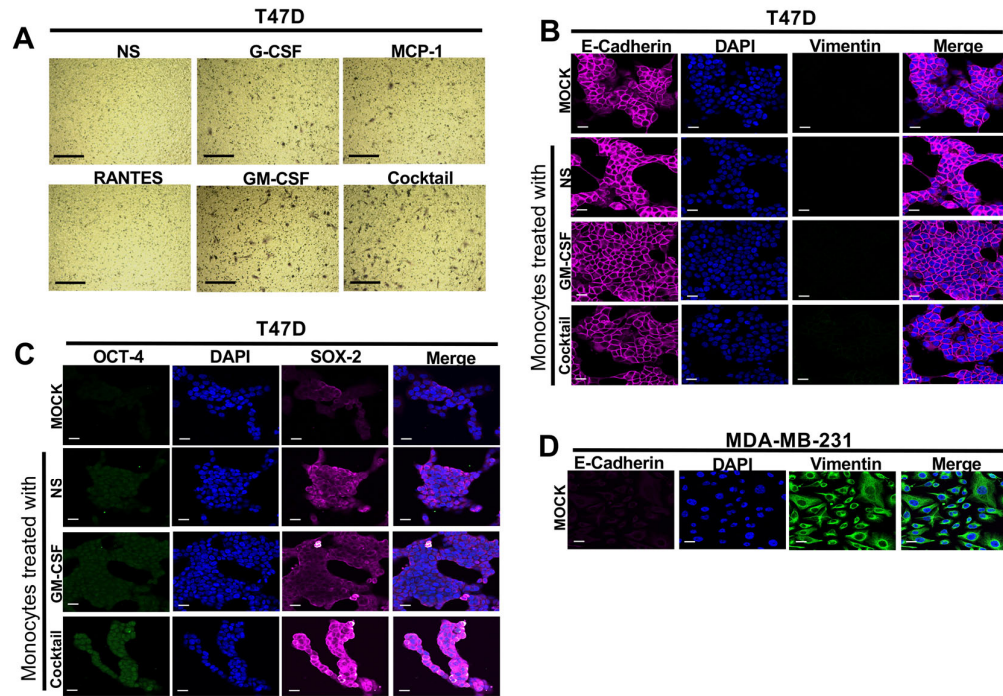


Figure S2. T47D cells representative images and vimentin positive staining. (A) Matrigel-based transwell invasion assay. The scale bars indicate 100 μ m, magnification 100 \times . (B) Immunofluorescence analysis of epithelial to mesenchymal transition and (C) of stemness markers. (B) E-cadherin (magenta), vimentin (green) and nuclei (blue); (C) OCT4 (green), SOX2 (magenta) and nuclei (blue). (D) MDA-MB-231 cells were used as positive control for vimentin staining. The scale bars indicate 20 μ m, magnification 60 \times .

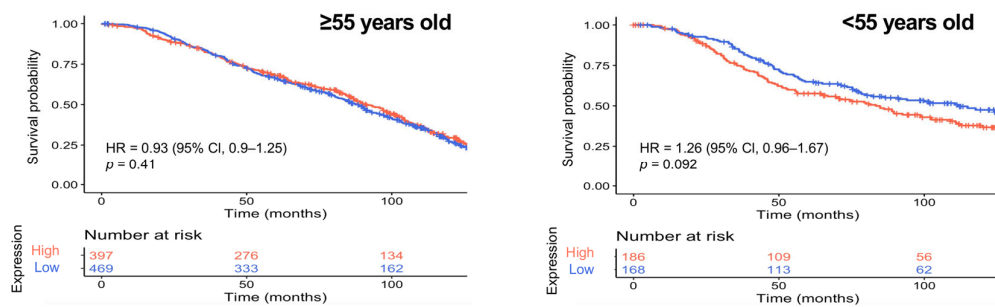


Figure S3. Breast cancer young patients with overexpression of any of GM-CSF, G-CSF, MCP1 or RANTES cytokines exhibit decreased survival. Kaplan-Meier survival curves of breast cancer patients divided by age, ≥ 55 and < 55 years old and comparing GM-CSF, G-CSF, MCP1 or RANTES over-expression (high, z-score > 1) against under-expression (low, z-score < -1). P-values were calculated using Log-Rank test and the hazard ratios (HR) were determined with 95% confidence intervals (95% CI).

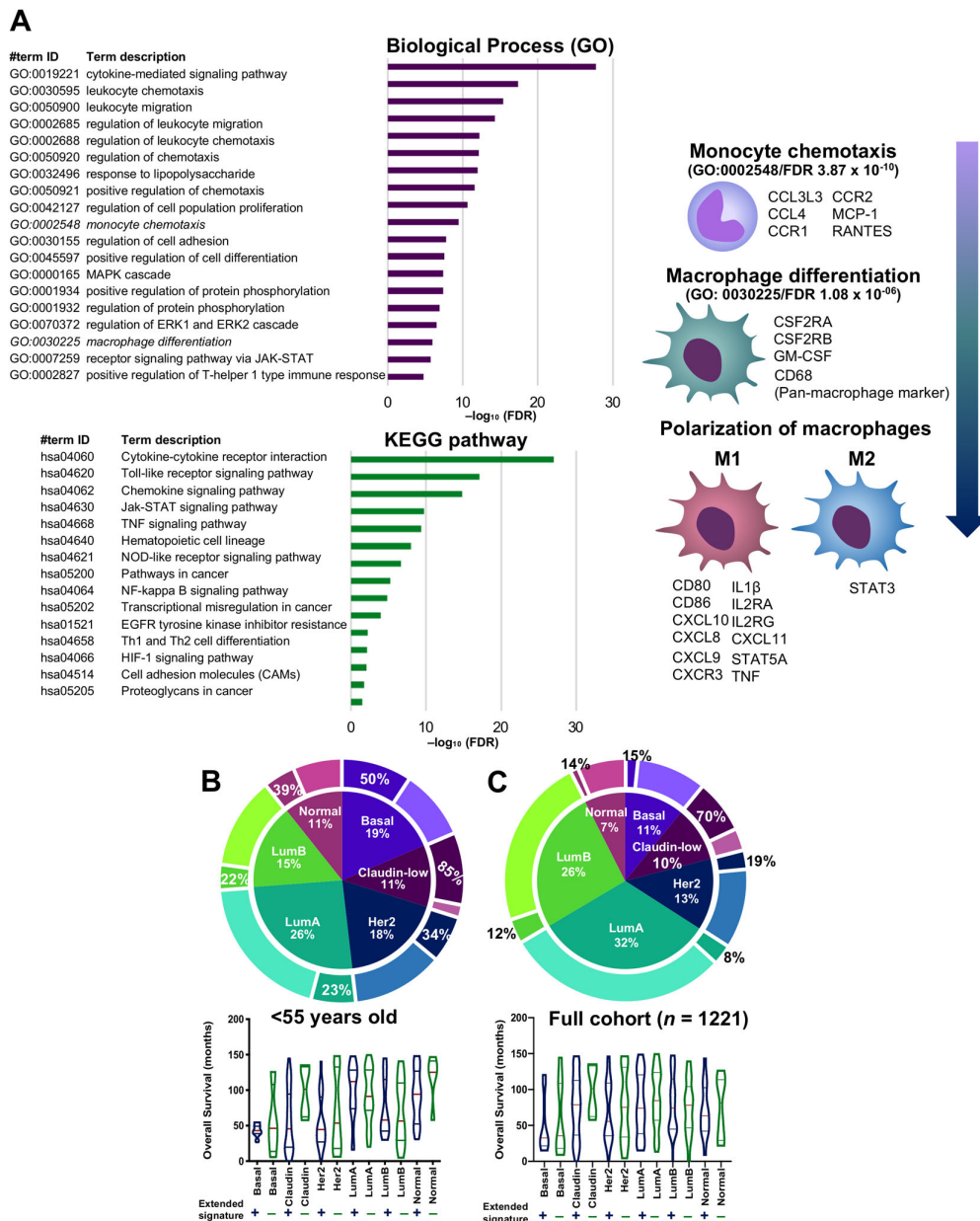


Figure S4. The extended gene expression signature is concordant with formation of M1-like macrophages and poor survival. (A) GO and KEGG pathway enrichment analyses with top terms related to the extended gene signature associated with GM-CSF, MCP1 and RANTES. Graphical summary of the top genes along the pathways of monocyte chemoattraction, macrophage maturation and polarization (right). GO term identity and FDR values are provided. Several terms included in the bar graph participate in the polarization of M1 and M2 macrophages, genes included in the signature that participate in this process were taken from the literature, since there is no GO or KEGG term that is specifically assigned. (B) Proportion of patients younger than 55 years old divided by subtype (includes claudin-low), outer ring representing in dark colors the proportion of samples positive (one SD above the median value of NES) to the extended signature, light colors represent the rest of the cohort. (C) Proportion of the full cohort of patients of METABRIC BRCA database divided by subtype that are positive (one SD above the median value of NES) to the extended signature. Below (B) and (C) pies, plots with the overall survival of patients with different BRCA intrinsic subtypes and that are positive and negative to the extended signature. Values from plots of (B) and (C) are shown in Table S2. NES: Normalized Enrichment Score, SD: standard deviation.

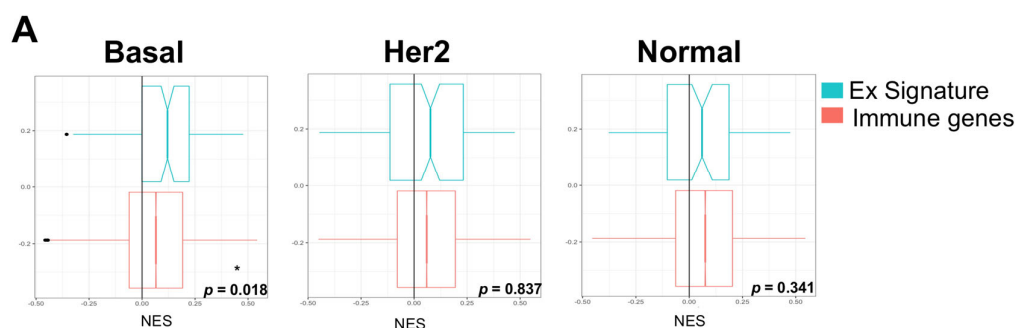


Figure S5. Permutation analysis comparing the M1-like macrophage extended signature and random genes related to immunity (A). Mann-Whitney-Wilcoxon test of NES values for basal, Her2 and normal subtypes. * $p < 0.05$. NES: Normalized Enrichment Score.

Table S1. Samples positive to over-expression of each cytokine (%)

| | G-CSF | MCP-1 | RANTES | GM-CSF |
|-------------|-------|-------|--------|--------|
| Basal | 15.04 | 22.56 | 30.08 | 27.07 |
| Claudin-low | 15.45 | 61.79 | 60.16 | 28.46 |
| Her2 | 11.88 | 12.5 | 25.63 | 16.88 |
| LumA | 18.18 | 7.32 | 8.08 | 8.33 |
| LumB | 16.35 | 9.75 | 8.8 | 14.78 |
| Normal | 17.58 | 26.37 | 20.88 | 19.78 |

Table S2. Summary of overall survival (months) data of patients of the different BRCA intrinsic subtypes that are positive and negative to the extended signature.

| Overall Survival (Months) | | | | | | | | | | | | |
|----------------------------------|--------------|--------------|----------------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------|---------------|
| Extended signature (<55 years) | | | | | | | | | | | | |
| | Basal (+) | Basal (-) | Claudin (+) | Claudin (-) | Her2 (+) | Her2 (-) | LumA (+) | LumA (-) | LumB (+) | LumB (-) | Normal (+) | Normal (-) |
| <i>n</i> | 13 | 4 | 34 | 4 | 22 | 10 | 19 | 20 | 12 | 17 | 10 | 5 |
| Med | 43.06 | 46.18 | 45.61 | 101.22 | 44.6 | 53.55 | 112 | 91.27 | 57.95 | 56.5 | 94.23 | 125.33 |
| Max | 54.69 | 125.7 | 144.67 | 135.33 | 140.33 | 148.07 | 147.77 | 149.6 | 144.77 | 140.77 | 148.03 | 146.83 |
| Min | 26.72 | 5.83 | 0.76 | 57.67 | 1.77 | 6.27 | 16.3 | 20.27 | 30.13 | 4.87 | 30.97 | 58 |
| SEM | 1.39 | 25.36 | 7.59 | 18.79 | 7.8 | 17.32 | 8.35 | 7.97 | 11.72 | 10.85 | 9.67 | 15.5 |
| Extended signature (full cohort) | | | | | | | | | | | | |
| <i>n</i> | 20 | 8 | 86 | 4 | 31 | 14 | 33 | 59 | 40 | 35 | 13 | 10 |
| Med | 32.58 | 35.65 | 78.93 | 101.22 | 55.63 | 75.22 | 74.1 | 84.63 | 74.2 | 78.16 | 63.5 | 81.58 |
| Max | 120.43 | 144.43 | 146.37 | 135.33 | 146.06 | 146.4 | 148.8 | 149.43 | 147.36 | 139.3 | 143.16 | 126.4 |
| Min | 15.3 | 9.13 | 0.77 | 57.67 | 1.77 | 4.43 | 15.2 | 13.4 | 2.53 | 1.27 | 9.06 | 21.9 |
| SEM | 8.3 | 18.32 | 4.53 | 18.79 | 7.43 | 13.33 | 7.49 | 5.19 | 6.18 | 6.28 | 10.27 | 13.41 |

Sample size (*n*), median (Med), maximum (Max), minimum (Min) and standard error of the mean (SEM). For this analysis, positive samples were considered those 1 standard deviation (SD) above the median of the NES (normalized enrichment score) value, while negative samples were 1 SD below the NES.

Table S3. Identity of the genes conforming all signatures and their expected up or down expression.

| Extended signature | | | | Th1 | | Monocyte/macrophages | | Claudin-low | | Immunosuppressive | |
|--------------------|---------------------|---------------|---------------------|---------------|---------------------|----------------------|---------------------|-------------|---------------------|-------------------|---------------------|
| Gene | Expected regulation | Gene | Expected regulation | Gene | Expected regulation | Gene | Expected regulation | Gene | Expected regulation | Gene | Expected regulation |
| CCL3L3 | UP | IL2RA | UP | CD4 | UP | FCN1 | UP | EPCAM | DOWN | CD274 | UP |
| CCL4 | UP | IL2RG | UP | DPP4 | UP | VCAN | UP | CLDN3 | DOWN | IDO1 | UP |
| CCR1 | UP | IL6 | UP | HAVCR2 | UP | CD14 | UP | CLDN4 | DOWN | FASLG | UP |
| CCR2 | UP | MCP1 | UP | IFNA1 | UP | CD33 | UP | CLDN7 | DOWN | CTLA4 | UP |
| CD68 | UP | RANTES | UP | IFNGR1 | UP | CSF3R | UP | CDH1 | DOWN | PDCD1 | UP |
| CD80 | UP | STAT3 | DOWN | IL2 | UP | CD68 | UP | SNAI1 | UP | LAG3 | UP |
| CD86 | UP | STAT5A | UP | KLRD1 | UP | CD80 | UP | MUC1 | DOWN | PDCD1LG2 | UP |
| CSF2RA | UP | TNF | UP | TNFSF11 | UP | CD86 | UP | TWIST2 | UP | IL10 | UP |
| CSF2RB | UP | | | IL-12A | UP | CXCR3 | UP | MME | UP | TGFB1 | UP |
| CXCL11 | UP | | | CCR1 | UP | GM-CSF | UP | ALDH1A1 | UP | PTGS2 | UP |
| CXCL10 | UP | | | GM-CSF | UP | RANTES | UP | ZEB2 | UP | | |
| CXCL8 | UP | | | IL10RA | UP | TNF | UP | TWIST1 | UP | | |
| CXCL9 | UP | | | RANTES | UP | STAT3 | DOWN | ITGB1 | UP | | |
| CXCR3 | UP | | | TNF | UP | | | SNAI2 | UP | | |
| GM-CSF | UP | | | | | | | ZEB1 | UP | | |
| IL10RA | UP | | | | | | | THY1 | UP | | |
| IL1B | UP | | | | | | | VIM | UP | | |

Genes in bold are genes shared by signatures