# Infiltration by CXCL10 secreting macrophages is associated with antitumor immunity and response to therapy in ovarian cancer subtypes

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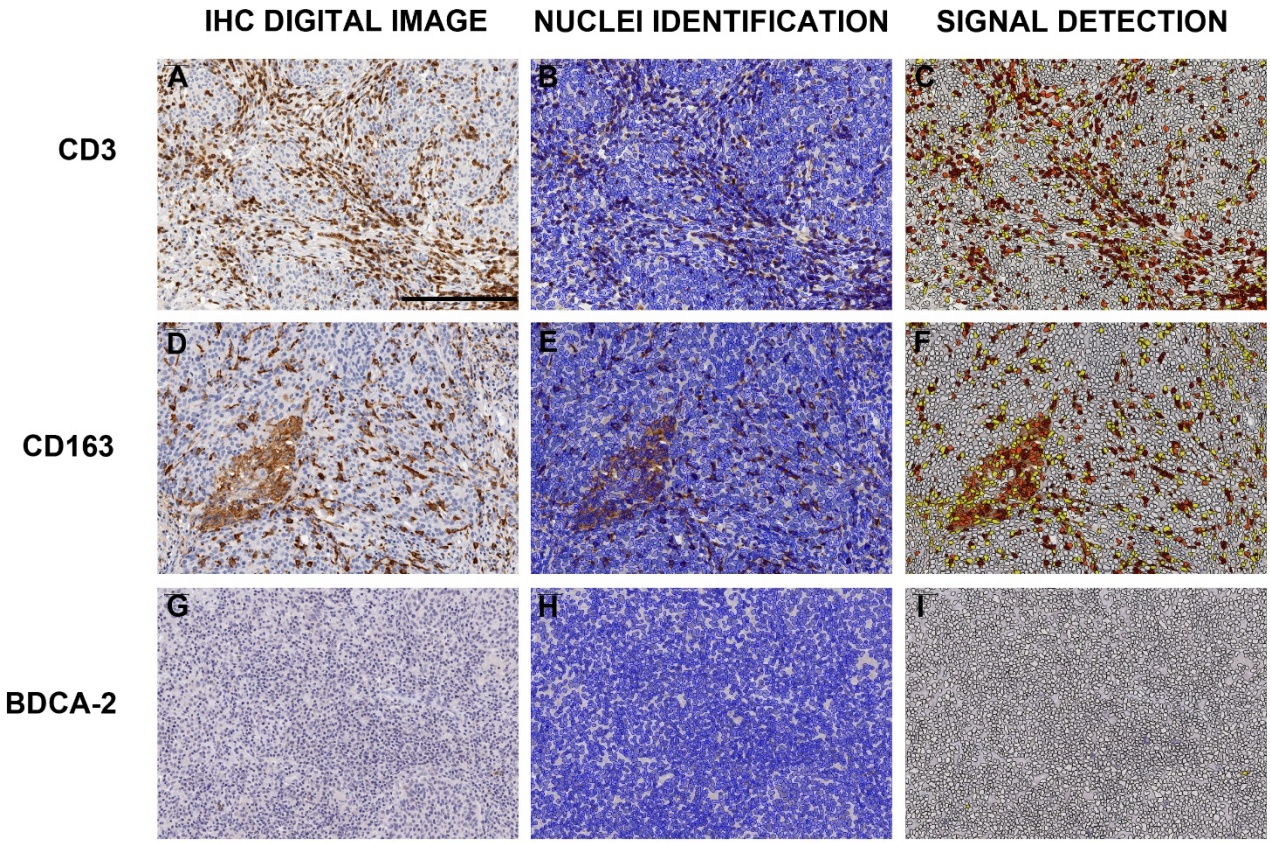
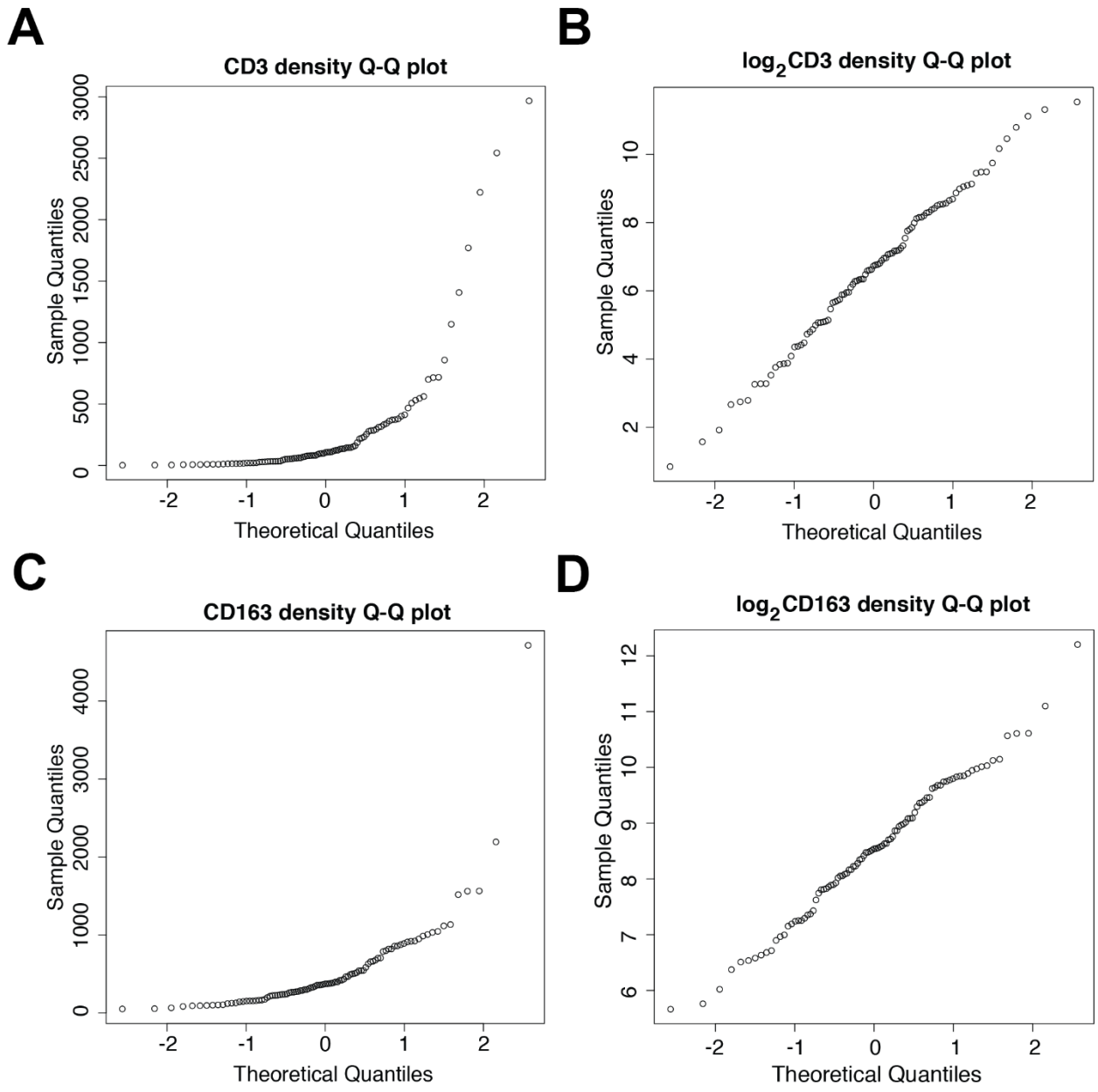
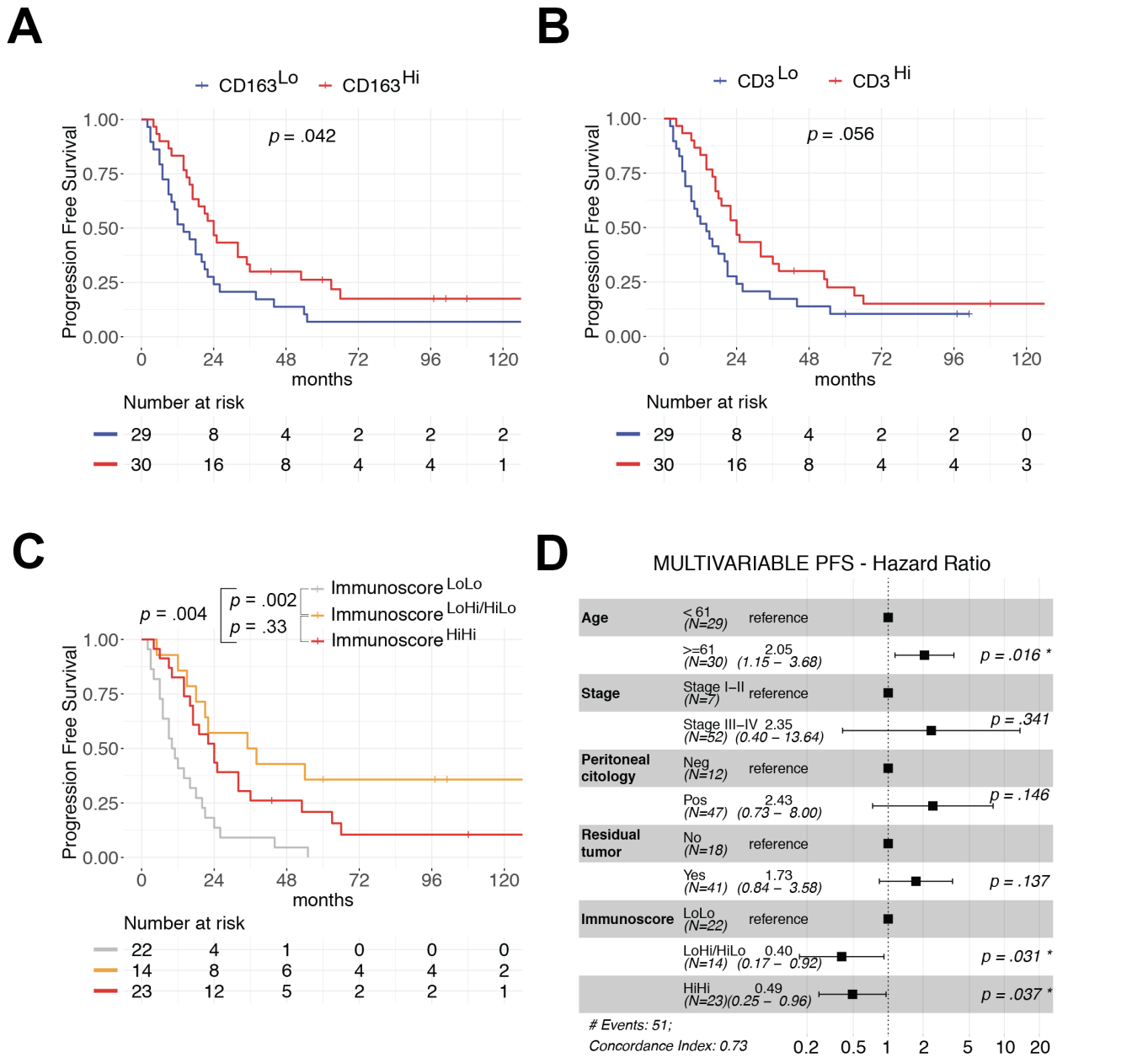
## Supplementary Figures



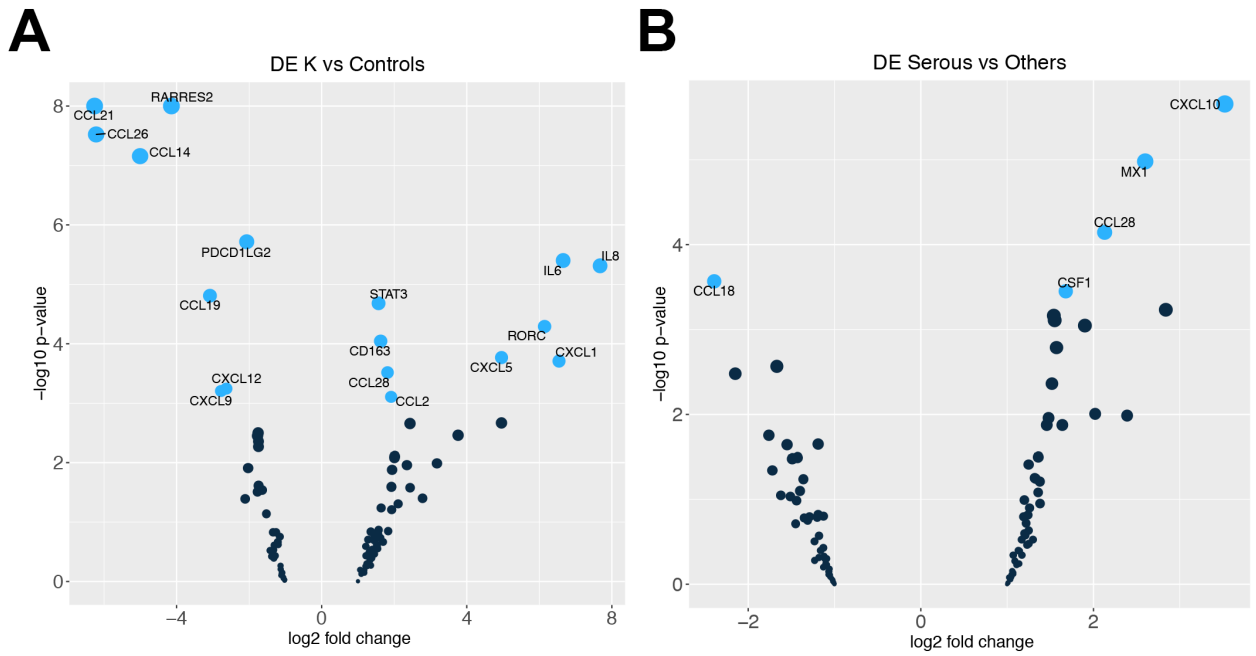

**Supplementary Figure S1. Digital image analysis of T-cells, tumor-associated macrophages (TAMs) and Plasmacytoid dendritic cells (PDCs) in ovarian carcinoma.** Sections are from one representative HGSC case. **A, D, G**: digitalized immunostained slides (200x magnification; scale bar 200 um) recognizing $CD3^+$ T-cells (**A**), $CD163^+$ TAMs (**D**) and $BDCA2^+$ PDCs (**G**). **B, C, E, F, H, I**: absolute immune cells count performed with Tissue Studio 2.0, based on segmentation of nucleus objects (**B, E, H**) and recognition of positive intraepithelial and stromal immune cells (**C, F, I**), with different staining intensity (yellow mild, red moderate and brown strong).



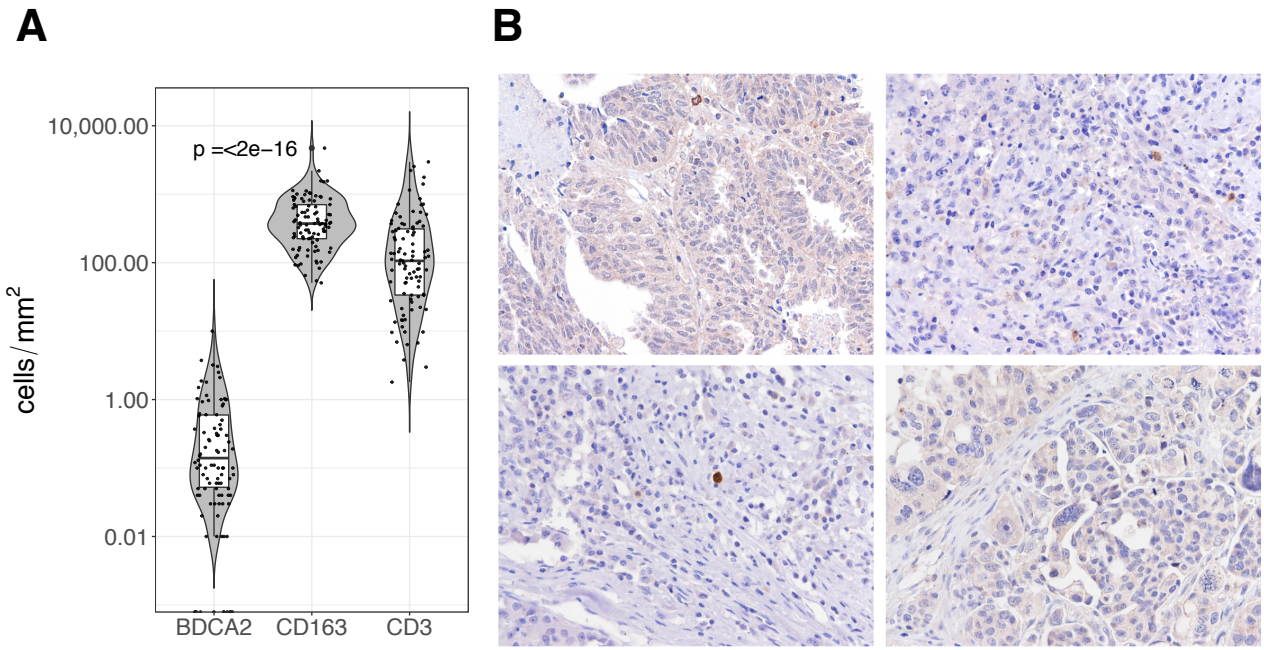

**Supplementary Figure S2**. **Distribution of immune cell variables.** Q-Q plots of $CD3^+$, $log_2CD3^+$, $CD163^+$ and $log_2CD163^+$ cells densities showing that both immune populations densities follow log-normal distributions.



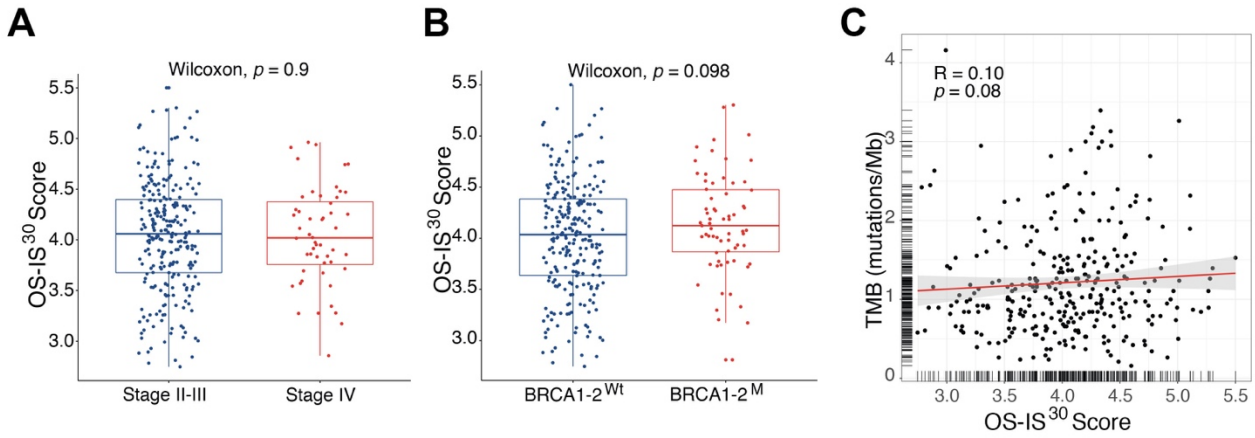

**Supplementary Figure S3**. **Progression free survival and immune contexture.** Univariable analysis of progression free survival estimates (Kaplan-Meier method) according to high density of $CD3^+$ T-cells (**A**), high density of $CD163^+$ TAMs (**B**) or Immunoscore classes (**C**). P values estimated with log-rank test (**A, B**), pairwise comparisons p-values adjusted with FDR (**C**). Forest-plot reporting H.R. with $CI_{95\%}$ of multivariable progression free survival analysis with Cox-model (**D**).



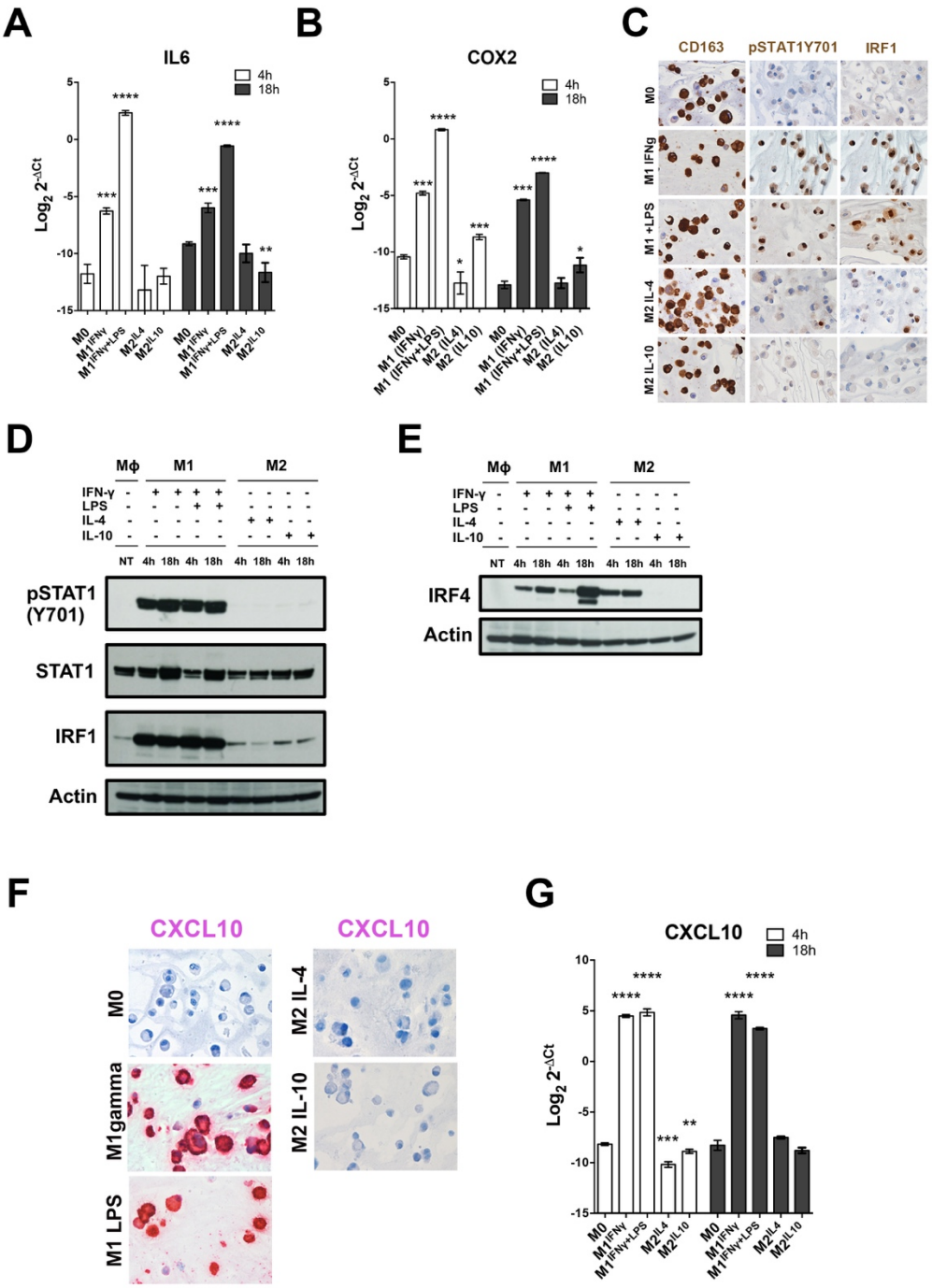

**Supplementary Figure S4. Differential gene expression analysis.** Volcano plots showing differentially expressed genes in patients affected by ovarian cancer, compared to healthy ovarian controls (**A**), and differentially expressed genes in HGSC compared to EC or CCC (**B**). Light blue dots representing significant different expressed genes by FDR adjustment.



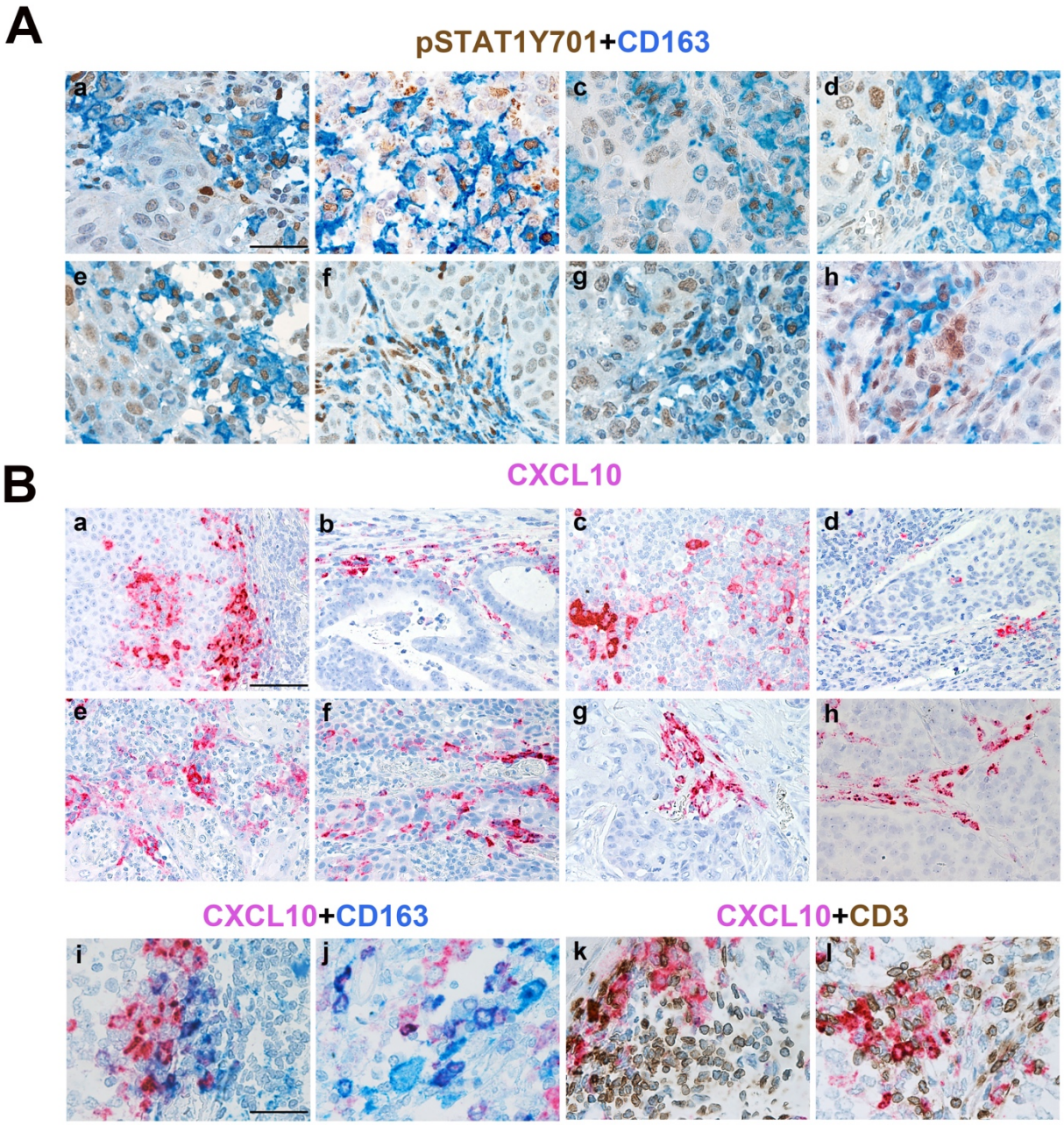

**Supplementary Figure S5. Limited infiltration of plasmacytoid dendritic cells in OCs.** Violin plots of immune cells distribution in OCs sections, p value estimated by ANOVA (**A**); representative sections from OCs cases immunostained for BDCA2 showing the rare presence of BDCA2$^{+}$ PDCs (**B**). Magnification 200x.



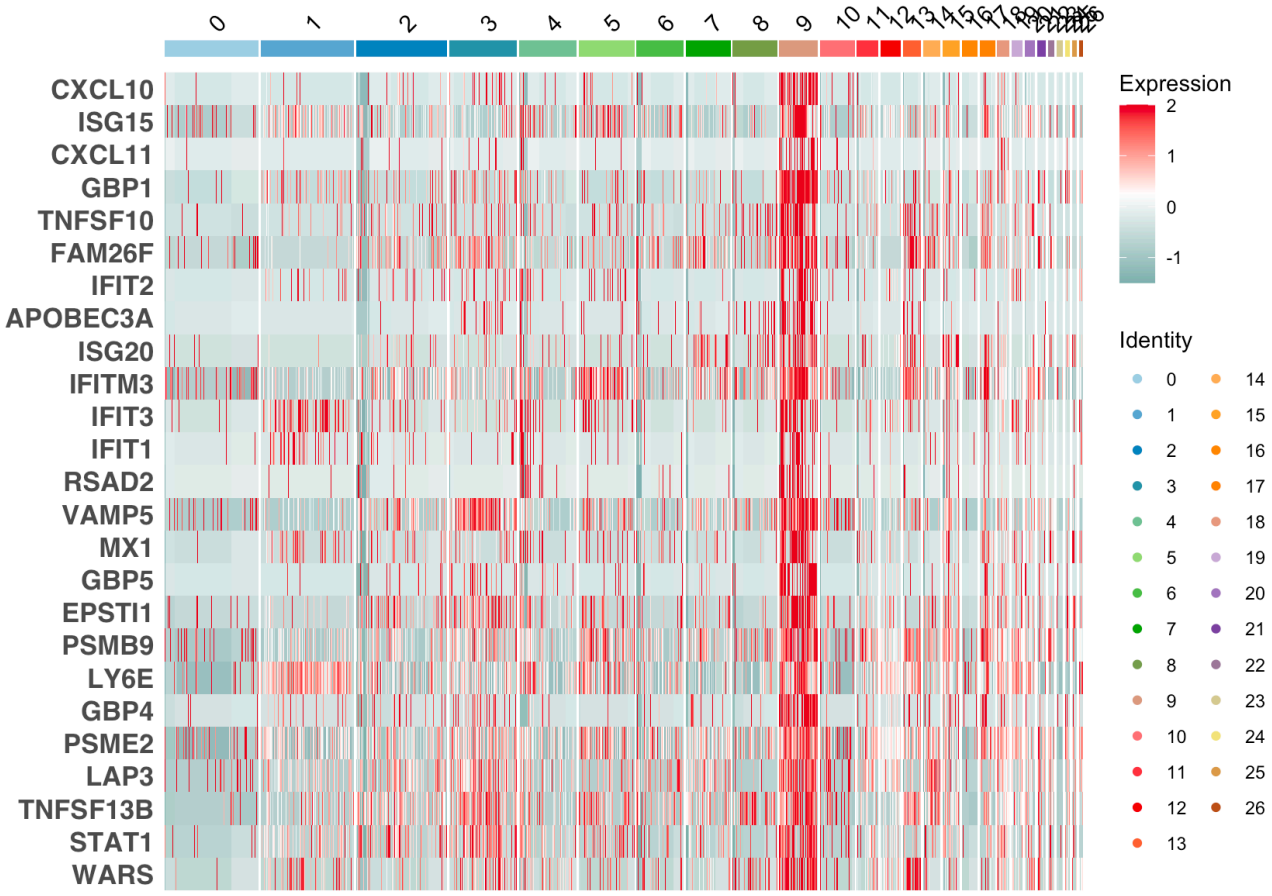

**Supplementary Figure S6. Clinical correlates of OC-IS$^{30}$.** Box plots showing OC-IS$^{30}$ score in different tumor stages groups (**A**), BRCA1 and BRCA2 mutational status groups (**B**) and scatter plot showing no correlation between OC-IS$^{30}$ score and TMB (**C**). P values estimated by Wilcoxon unpaired test in A, B and Spearman test in C.




**Supplementary Figure S7. Macrophage polarization in OCs.** In **A, B** and **G** the IL6, COX2 and CXCL10 genes expression was measured by qRT-PCR on polarized monocyte-derived Mϕ, IFNa (100 U/mL) unstimulated or stimulated with IFN-γ (50 ng/mL) or IFN-γ (20 ng/mL) + LPS (100 ng/mL) or IL-4 (20 ng/mL) or IL-10 (20 ng/mL) for 4 or 18 hours. The mRNA relative expression (-ΔCt) was normalized to the housekeeping gene using the comparative Ct method. The mean and SD of technical replicates (n = 3) are shown. The statistical significance was calculated by a Student's t test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. In **C** sections from cell-block preparations of polarized monocyte-derived Mϕ subjected to *in situ* hybridization as labeled. Magnification 400X. Western blot analysis performed on polarized monocyte-derived Mϕ illustrate the protein expression of IRF1, pSTAT1Y701 and IRF4 as labeled (**D, E**). Sections from cell-block preparations of polarized monocyte-derived Mϕ subjected to *in situ* hybridization as labeled. Magnification 400X (**F**).




**Supplementary Figure S8. M1-type Mϕ polarization across cancer types.** Sections from various cancer types (**A, B**) immunostained as labeled. Magnification 200X (B: a-h; scale bar 100 um) and 400X (A, B: i-l, scale bar 50 um). Sections from various cancer types showing the presence of $CD163^{+}pSTAT1Y701^{+}$ (**A**, **a** melanomas, **b** colorectal carcinoma, **c** endometrial carcinoma, **d** lung carcinoma, **e** oral squamous cell carcinomas, **f** laryngeal carcinoma, **g** triple negative breast carcinoma, **h** $HER2^{+}$ breast carcinoma). CXCL10 is abundantly produced in different tumor types (**B**, **a** melanomas, **b** colorectal carcinoma, **c** endometrial carcinoma, **d** lung carcinoma, **e** oral squamous cell carcinomas, **f** laryngeal carcinoma, **g** triple negative breast carcinoma, **h** $HER2^{+}$ breast carcinoma). and it is expression is detected in $CD163^{+}$ TAMs (**B: i** and **k** melanoma; **j** colorectal carcinoma, **l** endometrial carcinoma).




**Supplementary Figure S9: scRNAseq analysis of myeloid cells across cancer types.** Heatmap showing normalized expression of the top 25 most variable genes in cluster 9, as defined in Figure 9A.