Supplementary Materials

Supplementary Figure 1 Expression patterns of Siglec-9 ligand and Siglec-9$^+$TAMs in HGSC.

(A-B) Immunohistochemistry evaluation of Siglec-9 ligand expressing cells **(A)** and Siglec-9$^+$TAMs **(B)** in Fudan cohort (n = 120). **(C-D)** Fraction of patients with infiltration of Siglec-9 ligand expressing cells **(C)** and Siglec-9$^+$TAMs **(D)** according to FIGO stages. The Chi-square test was used to compare groups. **(E)** Expression of Siglec-9 ligands in HGSC cancer cell lines evaluated by flow cytometry. Histogram of Siglec-9 expression in SKOV3 cell line as analyzed by flow cytometry, non-stained cells in grey (left). Broad analysis of ligand expression to Siglec-9 on different tumor cell lines, as well as on PBMCs (n = 3) from healthy donors for comparison. Values were expressed as the mean fluorescence intensity (MFI) ratio of specific staining compared with isotype antibody only (right). **(F)** Expression of Siglec -9 ligands on HO-8190 HGSC cells was localized to the cell surface. Staining was lost upon neuraminidase treatment (sialic acid dependency). Original magnification, ×630.

Supplementary Figure 2 Gating strategy for flow cytometry assays to identify immune cells generally in fresh HGSC tissues.

(A) Representative plots showing the flow cytometry gating strategy to identify single cells **(B)** Representative plots showing the flow cytometry gating strategy for Siglec-9$^+$immune cells. **(C)** Representative flow cytometric plots of the gating strategy of cell composition of CD45$^+$ immune cells in tumor tissues of HGSC, including B cells (CD3$^-$CD20$^+$), T cells (CD3$^+$CD19$^-$), NK cells (CD3$^-$CD56$^+$), macrophages (CD68$^+$) and dendritic cells (DCs, CD11c$^+$).

Supplementary Figure 3 Mean fluorescence intensity (MFI) of biomarkers of M1 and M2 in Siglec-9$^+$TAMs of HGSC.

(A, C, E, G) Representative plots showing the flow cytometry analysis of mean fluorescence intensity (MFI) of M1-like (CD86 and HLA-DR) and M2-like (CD163, CD206, arginase-1 and PD-L1) in Siglec-9$^+$TAMs as compared to Siglec-9$^-$ TAMs in primary tumor tissues **(A)**, ascitic

28 fluid samples **(C)** from HGSC, in CD14$^+$monocytes derived from healthy donors were treated
29 with tumor conditioned media (TCM) **(E)** and ascites fluid **(G)** corresponding to control.
30 **(B, D, F, H)** Flow cytometry analysis of mean fluorescence intensity (MFI) of M1-like (CD86
31 and HLA-DR) and M2-like (CD163, CD206, arginase-1 and PD-L1) in Siglec-9$^+$TAMs as
32 compared to Siglec-9$^-$ TAMs in primary tumor tissues **(B)**, ascitic fluid samples **(D)** from
33 HGSC, in CD14$^+$monocytes derived from healthy donors were treated with tumor conditioned
34 media (TCM) **(F)** and ascites fluid **(H)** corresponding to control. Significance was assessed by
35 Mann-Whitney U test.
36
37 **Supplementary Figure 4** Single-cell sequencing data profiling of the fallopian tube, primary
38 and ascites HGSC tumor environments.
39 **(A)** Dimensionality reduction (UMAP) projections and clustering of 51,603 cells color-coded
40 for the indicated cell type from 28 samples (*n* = 17 patients). **(B)** Violin plots of marker gene
41 expression averaged per cluster, showing differential expression expressed genes in rows
42 and clusters in columns. **(C)** Heatmap showed the large-scale CNVs for epithelial cells (rows
43 along the y-axis) from 28 samples (*n* = 17 patients). CNVs were inferred according to the
44 average expression of 100 genes spanning each chromosomal position (x-axis). Red: gains;
45 blue: losses. Malignant HGSC cells from different patients and the range of different
46 chromosomes were indicated as different color bars on the left and top of the heatmap,
47 respectively. **(D)** Monocle2 trajectory analysis of monocyte/macrophage cells recapitulates
48 known lineage relationships, with classical monocytes (CD14$^+$) branching into macrophages.
49 **(E)** Heatmap showing the dynamic changes in gene expression along the pseudotime. The
50 distribution of macrophage subtypes during the transition, along with the pseudotime.
51 Subtypes were labeled by colors. **(F)** Gene Set Enrichment Analysis (GSEA) plots of metabolic
52 pathways were shown in Siglec-9$^+$TAMs compared to Siglec-9$^-$ TAMs. The signature was
53 defined by genes with significant expression changes. **(G)** The inferred IL-10 signaling
54 networks. Circle sizes were proportional to the number of cells in each cell group and edge

55 width represents the communication probability. **(H)** The differential genes that were
56 upregulated in Siglec-9$^+$TAMs compared with Siglec-9$^-$ TAMs from the integrated data (Figure
57 3D) were subjected to motif enrichment analysis. The top transcription factors from the
58 analysis were shown. **(I)** Significant signaling pathways were ranked based on differences in
59 the overall information flow within the inferred networks between primary tumors and
60 ascites. The overall information flow of a signaling network was calculated by summarizing
61 all communication probabilities in that network. The top signaling pathways colored red
62 were enriched in ascites, and colored green were enriched in the primary tumors. **(J)**
63 Comparison of the significant ligand-receptor pairs between primary tumor and ascites,
64 which contributed to the signaling from CD8$^+$T to Siglec-9$^+$TAMs subpopulations. Dot color
65 reflects communication probabilities and dot size represents computed *p*-values. Space
66 means the communication probability was zero. *p*-values were computed from one-sided
67 permutation test.
68
69 **Supplementary Figure 5** Siglec-9$^+$TAMs infiltration was associated with CD163$^+$, CD8$^+$T cells
70 and immune phenotype in Fudan cohort.
71 **(A-B)** Scatter plots showing the Spearman correlation analysis results between the score of
72 Siglec-9$^+$TAMs and M1-like **(A)** or M2-like **(B)** markers in HGSC TCGA specimens (*n* = 316).
73 The rug plots on the right of the y-axis show the expression of CD68 and the Siglec-9 on top
74 of the x-axis showed individual patients. Color scale: expression of markers from low (white)
75 to high (purple). **(C)** Association between Siglec-9$^+$TAMs and FIGO stage in HGSC TCGA
76 specimens (*n* = 316). The Chi-square test was used to compare groups. **(D)** Kaplan-Meier
77 curves of OS stratified by expression of Siglec-9$^+$TAMs score in HGSC TCGA cohort (*n* = 316)
78 using the Log-rank test. **(E)** Association between Siglec-9$^+$TAMs and CD163 in Fudan cohort
79 (*n* = 316). The Chi-square test was used to compare groups. **(F)** Kaplan-Meier curves of OS
80 stratified by infiltration of Siglec-9$^+$TAMs and CD163 in patients in Fudan cohort (*n* = 120)
81 using the Log-rank test. **(G)** Expression of GZMB$^+$IFN-γ$^+$ and GZMB$^+$TNF-α$^+$on CD8$^+$T cells

82 stratified by infiltration of Siglec-9$^+$TAMs. **(H)** Upregulated genes of both immunostimulators
83 and immune checkpoints in Siglec-9$^+$TAMs. *P* values were obtained by Bonferroni-corrected.
84 **(I-K)** Kaplan-Meier curves of OS stratified by CD8$^+$T infiltration in patients with stage III or IV
85 from Fudan cohort (n = 92) **(I)** as well as for low Siglec-9$^+$TAMs (n = 49) **(J)** and high
86 Siglec-9$^+$TAMs (n = 43) **(K)** proportion in patients with stage III or IV HGSC tumors using the
87 Log-rank test.
88

89 **Supplementary Figure 6** Siglec-9 blockade enhanced the antitumor activity of CD8$^+$T
90 leucocytes and skews macrophages to an M1-like phenotype.
91 **(A-C)** Intracellular cytokine production of (**A**) GZMB$^+$IFN-γ$^+$ or (**B**) GZMB$^+$TNF-α$^+$ or (**C**)
92 IFN-γ$^+$TNF-α$^+$ by healthy donor CD8 $^+$T cells co-cultured with TCM-educated macrophages in
93 direct or indirect contact upon engagement by α Siglec-9 (5 mg/mL) (n = 3-5, t-test). **(D-E)**
94 HGSC single cell suspension was incubated with control or Siglec-9-neutralizing antibody and
95 subjected to flow cytometric analysis to determine the expression of biomarkers of CD86,
96 HLA-DR **(D)** and CD163, CD206, Arginase-1, PD-L1 **(E)** stratified by Siglec-9$^+$TAMs
97 infiltration. Two-sided Wilcoxon rank-sum test was used for pairwise comparisons.
98

99 **Supplementary Figure 7** Gating strategy for flow cytometry assays to identify T cells and
100 macrophages in fresh HGSC tissues.
101 **(A)** Representative plots showing the flow cytometry gating strategy for M1 and M2
102 macrophages in Siglec-9$^+$TAMs. **(B)** Representative gating strategy for tumor-infiltrating CD8$^+$,
103 CD4$^+$Foxp3$^-$ Teff and CD4$^+$Foxp3$^+$Tregs, where expression of cytolytic markers was assessed
104 as well as co-inhibitory receptors.
105

106 **Supplementary Figure 8** Relationship between Siglec-9$^+$TAMs infiltration and ICB-response
107 signature predicting poorer responses HGSC TCGA cohort.

108 **(A-B)** Scatter plots showing the Spearman correlation analysis results between the score of
109 Siglec-9$^+$TAMs and DDIR (DNA Damage Immune Response), Immune checkpoint, Tertiary
110 lymphoid structure TLS, IFN signature 18 genes **(A)** and indicated signatures for good
111 prognosis responsive to ICB-treatment **(B)** in HGSC TCGA specimens (*n* = 316). The rug plots
112 on the right of the y-axis showed score of corresponding pathways and the Siglec-9$^+$TAMs
113 score on top of the x-axis showing for individual patients. *P* and r values were from a
114 Spearman correlation coefficient test.
115

116 **Supplementary Figure 9** Siglec-9$^+$TAMs and PD-L1 expression panel correlated with
117 molecular alterations and clinical outcome in HGSC.
118 **(A)** Forest plots of HR for overall survival high Siglec-9$^+$TAMs score versus the other were
119 respectively protracted according to distinct levels of PD-L1 expression. **(B)** Kaplan-Meier
120 curves of overall survival (OS) stratified by Siglec-9$^+$TAMs infiltration combing PD-L1
121 expression level in TCGA cohort (*n* = 316) using the Log-rank test. **(C)** Scatter plots showing
122 the Spearman correlation analysis results between the score of Siglec-9$^+$TAMs and *CD274*
123 expression (R=0.64, $P<0.05$) in patients of the TCGA cohort. The rug plots on the right of the
124 y-axis showed the expression of *CD274* and the Siglec-9$^+$TAMs signature defined on top of
125 the x-axis showed individual patients. Color scale: expression of markers from low (white)
126 to high (purple). Data were analyzed by Mann–Whitney U test and presented as mean and
127 SD. **(D)** Landscape of pathway enriched in molecular function and biological processes, along
128 with PD-L1 expression across four subgroups (Siglec-9$^+$TAMs^{low}PD-L1^{neg},
129 Siglec-9$^+$TAMs^{low}PD-L1^{pos}, Siglec-9$^+$TAMs^{high}PD-L1^{neg}, Siglec-9$^+$TAMs^{high}PD-L1^{pos}) in TCGA
130 cohort. **(E)** Landscape of genomic alterations and spectrum of inferred COSMIC mutational
131 signatures across four subgroups in TCGA cohort. Statistical significance (Mann-Whitney U
132 test $P < 0.05$) was indicated with asterisk. (*, $P < 0.05$) HRs were evaluated by univariate Cox
133 analysis in the TCGA cohort. HRR, homologous recombination repair; MMR, mismatch repair;

134 RTK, receptor tyrosine kinase; CI, confidence interval; HR, hazard ratio; GA, gene alteration;
135 WT, wild type.
136
137 **Supplementary Figure 10** Oncogenic pathway and immune pathway activity across
138 Siglec-9⁺TAMs and PD-L1 panel in HGSC.
139 **(A)** Pathway activity was estimated as the mean expression of downstream genes targeted by
140 each pathway. Only genes that were transcriptionally activated by these pathways were
141 considered. Kruskal-Wallis test p-values were Benjamini-Hochberg corrected. **(B-C)** Violin
142 plot showing portions of CD20 expression stratified by Siglec-9⁺TAMs and PD-L1 in Fudan
143 cohort (n = 120) **(B)** and B cell signature in HGSC TCGA cohort (n = 316) **(C).** In the box plots
144 inside violin plots the black horizontal lines represent the sample means, the boxes extend
145 from the first to third quartile and the whiskers indicate values at 1.5 times the interquartile
146 range. Data were analyzed by Kruskal-Wallis test. **(D)** Summary of molecular characteristics
147 found in the present study, and potential therapeutic implications for the treatment of HGSC
148 per subtype of infiltration of Siglec-9⁺TAMs combined with PD-L1. From top to bottom:
149 Siglec-9⁺TAMs subtypes, PD-L1 subtypes, shared genomic features among infiltration
150 subtypes; unique characteristics per infiltration subtype; suggested therapeutic strategies
151 per infiltration subtype.
152
153 **Supplementary Table 1.** Patient characteristics and relationship with Siglec-9⁺TAMs cell
154 infiltration.
155 **Supplementary Table 2.** Immunohistochemistry antibodies.
156 **Supplementary Table 3.** Flow cytometry antibodies and reagents.
157 **Supplementary Table 4.** Univariable and Multivariate analysis for OS in Fudan Cohort.
158 **Supplementary Table 5.** Univariable and Multivariate analysis for OS according to
159 Siglec-9⁺TAMs cells in Fudan Cohort.
160 **Supplementary Table 6.** The transcriptomic data were publicly available.

161

162 **Supplementary methods**

163 **HGSC patient tissue.**

164 *Preparation of single-cell suspensions.* Fresh tumor samples were processed
165 independently with enzymatic digestion and mechanical dissociation immediately after
166 collection to generate single-cell suspensions. Briefly, each tumor was cut into small pieces
167 with approximately 1-mm^3 in RPMI 1640 medium with 10% fetal bovine serum (FBS),
168 followed by enzymatic type IV (Signa) digestion for 90 min on a rotator at 37°C. The digested
169 mixture was passed through a 40 μm cell strainer to obtain dissociated cells. The filtered
170 mixture was centrifuged at 400 g for 5 min, and after removal of the supernatant, the
171 pelleted cells were resuspended in red blood cell lysis buffer (Invitrogen) and incubated at
172 room temperature for 10 min. After washing twice with PBS, the dissociated cells from the
173 tumor were resuspended in a cell staining buffer (Invitrogen), consisting of 1X PBS
174 supplemented with 0.04% BSA. Cells were collected from the ascites via centrifugation either
175 by paracentesis or during laparotomy. After the collection of the fluid, the fluid was
176 centrifuged for 10 minutes at 500 x g at 4°C) and residual red blood cells in pellets were lysed
177 using the same procedure abovementioned. PBMCs were isolated using a leukocyte
178 separation solution, following the manufacturer's instruction (HISTOPAQUE-1077;
179 Sigma-Aldrich). Briefly, 5 ml of fresh peripheral blood was collected in EDTA anticoagulant
180 tubes and subsequently transferred into the solution. After density gradient centrifugation
181 for 20 min at 400 x g, PBMCs settled at the interphase were carefully collected and washed
182 twice with PBS. Residual red blood cells were lysed using the same procedure
183 abovementioned.

184

185 *Tumor Conditioned Medium (TCM).* Tumor tissues were washed with PBS, then minced
186 and seeded on a petri dish in RPMI 1640 medium containing 10% FBS. After 48 hours of
187 culture, the medium was filtered through a 0.22 μm ultra-low protein binding filter and

188 collected as the conditioned medium. Ascitic fluid was centrifuged at 400 x g for 5 min and
189 filtered through a 0.22 μm ultra-low protein binding filter and stored at -80℃ for long-term
190 storage.
191
192 **Cell lines.** SKOV3, OVCAR-5, OVCAR8, A2780 and HO-8190 were acquired from ATCC. Cell
193 lines were tested for their authentication by STR-PCR, performed by Biowing (Shanghai,
194 China), before the start of the project. All the cell lines were cultured in RPMI 1640
195 supplemented with 10% Fetal Bovine Serum and 1000 U/mL Penicillin-Streptomycin, except
196 for SKOV3 in Mc5A (ATCC) supplemented with 10% Fetal Bovine Serum and 1000 U/mL
197 Penicillin-Streptomycin.
198
199 *Tumor lysate production.* SKOV3 tumor cells were harvested from 80% confluent culture
200 flasks and resuspended at $1x10^6$ cells/mL concentration for SKOV3 in PBS. Cells were
201 disrupted by five freeze-thaw cycles and subsequent sonication to produce a homogeneous
202 lysate.
203
204 **Immunohistochemistry (IHC).** Tissue microarray construction and the IHC protocol have
205 been described previously. The specimens were collected from the Obstetrics and
206 Gynecology Hospital of Fudan University within 30 min of the tumor resection and fixed in
207 10% formalin for 48 h. Dehydration and embedding in paraffin were performed as the
208 following routine methods. These paraffin blocks were cut into 5-μm sections and adhered to
209 a glass slide. Then, the paraffin sections were placed in the 70°C paraffin oven for 1 h before
210 being deparaffinized in xylene and then rehydrated in 100%, 90% and 70% alcohol
211 successively. The detailed procedure of immunohistochemistry was provided in Table S2.
212
213 *Single staining.* FFPE tissue was deparaffinized and antigen retrieval was performed
214 using citrate buffer (pH=6) and heat treatment. The tissue was blocked with Carbo-Free

215 Blocking Solution (Vector Labs) and then incubated with biotinylated Siglec-9 Fc chimera (5
216 μg/ml) for 30 min at room temperature, which was generated using a Mix-n-Stain biotin
217 labeling kit (Biotium). Slides were washed and incubated with Streptavidin-Peroxidase
218 conjugates for 30 min at room temperature. Development of section was performed with
219 3,3'-Diaminobenzidine (DAB, Abcam) and using hematoxylin as counterstain.
220
221 *Double staining.* FFPE tissue was performed on the human sample using a Double IHC
222 Kit (ZSGB-BIO DS-0006) according to the provided factory instructions.
223
224 *Pathologist Scoring.* Immunohistochemistry sections were scanned by Olympus CDD
225 camera, Nikon eclipse Ti-microscope (200X magnification) and NIS-Elements F3.2 software.
226 Qupath was used for quantification of the signal intensity of the ductal cells using the color
227 deconvolution plug-in. Two genitourinary pathologists, masked to the follow-up data, count
228 the number of positive staining cells at 200× magnification, and the average number was
229 used as the final number.
230
231 **Immunofluorescence (IF).** Siglec-9 ligand expression on OCT-embedded tissue sections
232 was assessed by lectin immunohistochemistry. Fresh frozen ovarian tumor sections were
233 cut at 10μm thickness and then fixed with the mixture of methanol /acetone (50/50, v/v),
234 solution and following washing steps. Blocking with BSA and Streptavidin/Biotin Blocking Kit
235 (Vector Labs). Sections were incubated overnight at 4°C with Siglec-9 Fc chimera (ligand)
236 and were subsequently detected with PE-streptavidin (Biolegend). Tissue sections were
237 counterstained with DAPI (Sigma-Aldrich) and quenched autofluorescence signal by
238 autofluorescence Quenching kit medium (Vector Labs), mounted in antifade mounting
239 medium (Vector Labs).
240
241 **Flow Cytometry.** Cryopreserved single-cell suspensions from human tumor tissue and cells

242 in ascitic fluid (and PBMCs for use as controls) were thawed. When indicated, cells were
243 treated with 25mU/mL of neuraminidase from Clostridium perfringens (Sigma-Aldrich) for
244 30 min at 37 °C to study the dependency of Siglec on the interaction with sialic acid. Live
245 single cells were sub-gated by staining with Zombie Aqua™ Fixable Viability Kit (1:500
246 dilution, Biolegend) for 30 minutes at room temperature in the dark. For blocking of Fc
247 receptors, cells were stained for 10 minutes on ice before immunostaining. After one wash
248 with flow cytometry buffer, cells were incubated with appropriate dilutions of various
249 combinations of the following antibodies. Appropriate antibody concentrations were
250 determined previously by titration in Table S3. Samples were fixed per manufacturers'
251 directions for 60 min (eBioscience) and stained for intracellular targets in 1X
252 permeabilization buffer (eBioscience), at 25°C for 60 minutes in the dark. Samples were
253 washed with PBS +2% FBS and resuspended in 1X stabilizing fixative for flow cytometry (BD)
254 use. The stained cells were acquired by a Beckman Coulter cytoflex flow cytometer using
255 FACS CytExpert software (Beckman Coulter) and the data were analyzed with the FlowJo
256 software (FlowJo LLC) v.10.7.2. All gates were set based on isotype control antibodies after
257 appropriate compensation using single-stained compensation controls.
258 *Phosphorylation analysis.* The phosphorylation of Siglec receptors induced by the tumor
259 lysates was analyzed using flow cytometry. $CD14^+$ isolated monocytes were aliquoted into
260 24-well U-bottom plates and 100 ng/mL M-CSF was added to cells to desired dilutions as
261 indicated for 3 days. Then educated macrophages with TCM or ascites and then treated with
262 anti-Siglec-9 or isotype control (both 5 mg/mL) in complete RPMI for 4 h at 4 °C.
263 Phosphorylation status of Siglec-9^+TAMs cells from healthy donors treated with tumor lysates
264 for 1 or 5, 10, and 15 minutes compared with time-matched controls (ratio) measured by
265 cytometry. The treatment was stopped by fixing cells at room temperature (RT) immediately
266 with 4% PFA (2% final volume)[1]. Cells were stained with antibodies according to
267 manufacturer protocol against cell surface markers and live/dead dye. After fixation with 4%
268 PFA for 10 minutes, cells were washed and resuspended in 150 uL of prechilled True-Phos™

269 Perm Buffer according to the manufacturer's protocol (Biolegend). Cells were then stained
270 with antibodies against intracellular markers (pSHP-1 and pSHP-2; 45 minutes to 1 hour at
271 RT) and analyzed on the flow cytometry the same day. All gates were set based on isotype
272 control antibodies after appropriate compensation using single-stained compensation
273 controls.
274
275 **In vitro flow-based phagocytosis assay.**
276 For all flow-based in vitro phagocytosis assays, tumor cells and Siglec-9^{+}TAMs were
277 co-cultured at a ratio of 2:1 in ultra-low-attachment 96-well U-bottom plates (Corning) in
278 serum-free RPMI (Thermo Fisher Scientific). PBMC-derived macrophages were
279 pre-incubated with a tumor-conditioned medium for 24-48 hours and separated
280 Siglec-9^{+}TAMs by fluorescence-activated cell sorting. HO-8190 tumor cells were labeled with
281 CFSE (Invitrogen) by suspending cells in PBS (2.5 μM working solution) for 25 min at 37°C
282 protected from light and washed twice with 20 ml of FBS-containing media before co-culture.
283 Carboxylate-modified red fluorescence latex beads with a mean diameter of 2 μm beads and
284 human macrophages were co-cultured in ultra-low-attachment 96-well U-bottom plates
285 (Corning) in serum-free RPMI (Thermo Fisher Scientific) at a bead: cell ratio of 1:500 for 1 h
286 at 37 °C and 5% CO2. For blocking binding with anti-Siglec-9, educated macrophages were
287 treated with 5 μg/mL anti-Siglec-9 or isotype control before being incubated with lysates for
288 4 h at 4 °C and then stimulated in the presence of lysates for 5min in room temperature.
289 Plates were washed two times; human macrophages were added to the plate; and plates were
290 incubated for 1h at 37°C Phagocytosis was stopped by washing with 4°C PBS and
291 centrifugation at 400 x g before the cells were stained with Live/Dead stain and anti-CD11b.
292 Assays were analyzed by flow cytometry, and phagocytosis was measured as the number of
293 CD11b^{+} and CFSE^{+} macrophages, quantified as a percentage of the total CD11b^{+}macrophages
294 and normalized to the control condition.
295

296 **Macrophages-mediated T-cell suppression assay**

297 To measure the T cell suppression by macrophages, macrophages were pre-incubated with a
298 tumor-conditioned medium for 24-48 hours and separated Siglec-9$^+$TAMs by
299 fluorescence-activated cell sorting. CD8$^+$T cells were isolated from peripheral blood using
300 MACS CD8 MicroBeads (Miltenyi). For blocking binding with anti-Siglec-9, Siglec-9$^+$TAMs
301 were treated with 5 μg/mL anti-Siglec-9 or isotype control before being incubated with
302 lysates for 4 h at 4 °C and then stimulated in the presence of lysates for 5min in room
303 temperature. Then macrophages (3 x 10^4 cells) were co-cultured with T cells (1.5 x 10^4 cells)
304 in the presence of anti-CD3/CD28 beads (Dynabeads Human T-Activator CD3/28, Thermo
305 Fisher Scientific) for 3 days at 37°C. Activated T cells without macrophages were used as a
306 positive control. T-cell proliferation was assessed by flow cytometry. Protein transport
307 inhibitor cocktail and cell stimulation cocktail (Invitrogen) was added to detect intracellular
308 cytokines in CFSE-labeled CD8$^+$T cells during the final 5 h. CFSE dilution was analyzed to
309 assess T cell proliferation.

310

311 **Ex vivo tumor stimulation assay.** To recapitulate the tumor faithfully from their derivation
312 and test the sensitivity to PD-1 blockade, we developed HGSC short-term organoids culture.
313 The single-cell suspension was incubated in 1x Red Blood Cell Lysis buffer (Biolegend) for
314 five minutes at room temperature and spun for three minutes at 1500 RPM. The lysis buffer
315 was aspirated and resuspended in RPMI-1640 (10% FBS, and 1% Pen/Strep). The
316 appropriate cell number of single cell suspension was diluted to a concentration of 6 x 10^6
317 cells/mL in RPMI-1640, 10% FBS, 1% Pen/Step, and 30 ng/mL of IL-2 (Peprotech) mixed
318 with 15% Matrigel (Corning). 40 uL of suspension was added per well of 24 well plates.
319 Anti-PD-1 antibody (Biolegend) and isotype control were with a final concentration of 5
320 μg/mL, neutralizing antibodies for Siglec-9 (R&D Systems) and isotype control (R&D Systems)
321 with a final concentration of 5 μg/mL antibody was used in RPMI-1640, 10% FBS, 1%
322 Pen/Strep, and 30 ng/mL of IL-2 for 96 hours. Protein transporter inhibitor (Biosciences)

323 was added to the media at a concentration of 1:500 and incubated for 5 hours before being
324 harvested for flow cytometry analysis. For intracellular T effector cytokine and cytotoxic
325 granule detection, cells were fixed and permeabilized (fixation and permeabilization kit,
326 eBioscience) as per the manufacturer's instructions.
327
328 **Genomic analysis and variant assessment.** Gene alterations (GA) were defined as the
329 aggregation of gene mutation and copy number variation (CNV). Either nonsense, missense,
330 frameshift, or splice-site variants affecting consensus nucleotides; on deleterious
331 homozygous deletions and amplifications were defined as gene alteration. The mutational
332 pattern of each sample was established by categorizing SNVs according to their
333 96-trinucleotide context. The contribution of each of the 79 mutational signatures from
334 COSMIC v3.3 (as deposited on June 2022).
335
336 **Single-cell RNA-seq analysis.** The scRNA-seq data was downloaded from the GEO
337 GSE151214[2], GSE154600[3] and GSE146026[4] as pre-processed raw data and imported into
338 the package Seurat (v4) for down-stream analysis (Table S6). The data were filtered to include
339 genes that were expressed in at least 25% of cells and cells that expressed at least 200 genes
340 and 3% ribosomal transcripts, not more than 6000 genes and less than 15% of
341 mitochondrial transcripts. Cell cycle effects were adjusted by regressing out the G2M and S
342 phase gene expression scores using the ScaleData function of the Seurat package. Doublets
343 were artefactual libraries generated from two cells arising due to errors in droplet
344 encapsulation of cells, and thus commonly affect the quality of single-cell sequencing data.
345 The R package "DoubletFinder"(https://github. com/chris-mcginnis-ucsf/DoubletFinder)
346 was applied to predict doublets in our data. A doublet was defined as a single-cell library
347 representing more than one cell, and a closer examination of some known markers would
348 suggest that the offending cluster consists of doublets of more than one cell type, while no cell
349 type was known to strongly express both markers at the same time. We removed doublets in

350 each sample individually, with an expected doublet rate of 0.04 and default parameters used
351 otherwise. The remaining cells that survived the filtering criteria were single. Then the gene
352 expression matrices for all cells from the fallopian tube, primary tumors and ascites were
353 combined and converted to a Seurat object using the FindIntegrationAnchors function of the
354 Seurat package. The SCTransform function was used to normalize and scale the data,
355 regressing out the mitochondrial percentage,and Principle component analysis (PCA) was
356 performed using the highly variable genes that were identified by the SEURAT function
357 "FindVariableGenes()". From the remaining cells, the tSNE transformation was performed
358 using the RunTSNE with default perplexity value [5]. These clusters were projected onto
359 Uniform Manifold Approximation and Projection (UMAP) dimensional reduction. Gene
360 expression matrices were generated with log normalization and linear regression using the
361 "NormalizeData()" and "ScaleData()" functions of the Seurat package. Cell populations were
362 identified using the SEURAT "FindClusters()" function with a resolution set to 1.5. Marker
363 genes for each cluster were identified using the SEURAT function "FindMarkers()" with
364 default parameters. The binary expression plots were generated by coloring the tSNE plot
365 with the expression status of selected genes, i.e. expressed (UMI count >0) or not expressed
366 (UMI count = 0).
367
368 *Cell cluster annotation.* Cell clusters were identified using the FindClusters function in
369 Seurat, with a K parameter of 20 and default parameters used otherwise. We annotated the
370 clusters as different major cell types based on their average gene expression of well-known
371 markers, including $CD4^{+}$T cell (PTPRC, CD3D, and CD4), $CD8^{+}$T cell (PTPRC, CD3D, and
372 CD8A), myeloid cell (CD14 and ITGAX encoding CD11C), macrophages (FCGR2A, CSFIR),
373 $CD14^{+}$monocytes (S100A9, CD14), malignant cell (EPCAM and KRT family genes), dendritic
374 cells (FLT3, IL3RA), B cell (CD19 and MS4A1), plasma cells (SLAMF7, IGKC), mast cells
375 (TPSAB1 and TPSB2), cancer-associated fibroblasts (PDPN and DCN) and stromal cells
376 (PECAM1, ACTA2). Clusters were also confirmed by identifying differentially expressed

377 marker genes for each cluster and comparing them to known cell-type-specific marker
378 genes.
379
380 *InferCNV analysis.* To identify malignant cells, we identified evidence for somatic
381 alterations of large-scale chromosomal copy number variants, either gains or losses, in a
382 single cell using inferCNV (https://github.com/broadinstitute/inferCNV), in addition to the
383 expression of EPCAM. The raw single-cell gene expression data were extracted from the
384 Seurat object according to the software recommendation. The single-cell data derived from
385 cancer-associated fibroblasts and stromal cells were included as a control reference. We
386 performed inferCNV analysis with the default parameters.
387
388 *Derivation and experimental validation of single-cell signatures.* As described in Figure
389 S4A-B, we sought to derive signatures for single cell populations (in particular, the
390 Siglec-9$^+$TAMs population) for application to bulk RNA-seq data and/or data from clinical
391 cohorts. Gene sets specific for each myeloid population were generated using the function
392 FindMarkersAll in the Seurat package to find differential genes expressed between each
393 cluster. To enable the discovery of highly specific single-cell signatures, we performed
394 multiple rounds of differential expression (DEG) analysis. First, we performed a
395 ''subpopulation'' DEG analysis, whereby each cluster (e.g. Siglec-9$^+$TAMs) was compared to
396 all other cells in its broad lineage (e.g. all other macrophage clusters). This round of DEG
397 establishes the DEGs specific to the cluster (i.e. subpopulation). For each cluster, we then
398 retained only those genes (log FC > 0.55; FDR P < 0.01) exclusive to that cluster's
399 subpopulation DEG. Subsequently, for each cluster, we performed a ''lineage'' DEG analysis, in
400 which each cluster (i.e. Siglec-9$^+$TAMs) was compared to all other clusters of other lineages
401 (e.g. all CD8$^+$clusters, and all myeloid clusters). For each subpopulation (e.g. Siglec-9$^+$TAMs)
402 within a lineage (e.g. CD8$^+$T cells, CD4$^+$T cells, TAMs, monocytes, B cells, Mast cells and
403 CD45-cells), we retained the same set of lineage genes, i.e. the set of genes that were

404 commonly upregulated across all subpopulations of the same lineage when performing DEG
405 analyses vs other lineages. As with the subpopulation DEG analysis, for each lineage, we only
406 retained genes that were exclusive to that lineage when compared to all other lineage
407 signatures. To define the final signature genes for each cluster, we combined its
408 subpopulation and lineage DEGs—and, to ensure that genes in each signature were only
409 expressed on immune cells and not on CD45- cells, we filtered out genes that were expressed
410 with FPKM >25 in the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle),
411 following a similar step as in Liu et al Nature 2020 [6]. We next sought to validate the specificity
412 of these signatures in an independent dataset of HGSC patients receiving bulk RNA
413 sequencing. The populations used for sorting and validation were described in subsequent
414 sections in the methods. Generating gene sets were later used in survival analysis using bulk
415 transcriptomics. These genes were also considered signature genes for each cluster and used
416 for bulk RNA-seq analysis as described in Figure 4G-H, Figure S5A-F and Figure 5H.
417
418 *Pathway enrichment analysis.* To gain functional and mechanistic insights into a cell
419 cluster, we performed Gene Ontology (GO) pathway enrichment analyses using the R
420 package clusterProfiler to identify biological pathways that were enriched in a certain gene
421 list more than that would be expected by chance. For non-malignant cells, the gene list
422 included the DEGs with IogFC > 0.25 and P value <0.05 in clusters. P value <0.05 was
423 considered to be a significant enrichment. To compare the difference in signaling pathway
424 enrichment between two clusters (Sigelc-9^{+}TAMs versus Sigelc-9^{-}TAMs), we performed the
425 gene set enrichment analysis (GSEA; version 3.0) using the selected molecular signatures
426 database v7.070. To explore the heterogeneous expression of TAMs, we performed gene set
427 variation analysis (GSVA, version 1.34.0), using 29 functional gene expression signatures
428 (Fges)[7] described in the molecular signature database.
429
430 *Motif enrichment analysis.* Motif enrichment analyses were performed using Metascape[8].

431 The motif enrichment analysis from Metascape was based on the TRRUST algorithm.

432

433 *Developmental trajectory inference.* To characterize the potential process of immune cell
434 functional changes and determine the potential lineage differentiation among diverse
435 immune cells, we performed trajectories analyses for monocytes and macrophages, using
436 Monocle2[9] (version 2.8.0; http://cole-trapnell-lab.github.iol/monocle-release/monocle2/).
437 The data of the indicated clusters calculated in Seurat was fed directly into Monocle2. Next,
438 we carried out density peak clustering (Monocle2 dpFeature procedure) to order cells based
439 on the genes with differential expression between clusters, using the differentialGeneTest
440 function in Monocle2. Genes with a q-value < 1e-4 were used to order the cells in pseudotime
441 analysis were used for ordering in all instances. The dimensional reduction was performed
442 by the Monocle2 function reduceDimension using the DDRTree method and cell order with
443 the default parameters of Monocle2.

444

445 *Receptor-ligand interaction analysis.* For malignant cells and myeloid clusters, we
446 identified all significant pair-wise interactions using CellChat[10]. Using the 6 myeloid clusters,
447 we generated the required metadata and count files (using log-normalized counts). For
448 CellChat analysis, this cleaned object was fed into the CellChat workflow, using the built-in
449 human ligand-receptor database, and tri-mean thresholding for the significance of
450 interaction.

451

452 **Reference**

453 1. Gough MJ, Melcher AA, Ahmed A, et al. Macrophages orchestrate the immune response to
454 tumor cell death. *Cancer Research* 2001;61(19):7240-47.
455 2. Dinh HQ, Lin X, Abbasi F, et al. Single-cell transcriptomics identifies gene expression
456 networks driving differentiation and tumorigenesis in the human fallopian tube. *Cell*
457 *reports* 2021;35(2) doi: 10.1016/j.celrep.2021.108978

458 3. Geistlinger L, Oh S, Ramos M, et al. Multiomic analysis of subtype evolution and
459 heterogeneity in high-grade serous ovarian carcinoma. *Cancer research*
460 2020;80(20):4335-45. doi: 10.1158/0008-5472.CAN-20-0521
461 4. Izar B, Tirosh I, Stover EH, et al. A single-cell landscape of high-grade serous ovarian cancer.
462 *Nature medicine* 2020;26(8):1271-79. doi: 10.1038/s41591-020-0926-0
463 5. Hao Y, Hao S, Andersen-Nissen E, et al. Integrated analysis of multimodal single-cell data.
464 *Cell* 2021;184(13) doi: 10.1016/j.cell.2021.04.048
465 6. Liu M, Kuo F, Capistrano KJ, et al. TGF-beta suppresses type 2 immunity to cancer. *Nature*
466 2020;587(7832):115-20. doi: 10.1038/s41586-020-2836-1 [published Online First:
467 2020/10/23]
468 7. Ng SS, Leonardelli S, Hölzel M. A pan-cancer fingerprint: common molecular denominators
469 of the human tumor microenvironment. *Signal Transduct Target Ther* 2021;6(1):394.
470 doi: 10.1038/s41392-021-00814-x
471 8. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the
472 analysis of systems-level datasets. *Nature Communications* 2019;10(1):1523. doi:
473 10.1038/s41467-019-09234-6
474 9. Trapnell C, Cacchiarelli D, Grimsby J, et al. The dynamics and regulators of cell fate
475 decisions are revealed by pseudotemporal ordering of single cells. *Nature*
476 *biotechnology* 2014;32(4):381-86. doi: 10.1038/nbt.2859
477 10. Jin S, Guerrero-Juarez CF, Zhang L, et al. Inference and analysis of cell-cell communication
478 using CellChat. *Nature Communications* 2021;12(1):1088. doi:
479 10.1038/s41467-021-21246-9