**Supplemental material**

**Flow cytometry**

For flow cytometry analysis, cells were suspended in 1 mL PBS with 2% FCS (Biochrom). Cell suspension was incubated with the different fluorophore-coupled primary antibodies for 20 min on ice. The following antibodies were used: CD45 (552848), CD11b (552850) and CD4 (560468) and CD8 (557654) from BD Biosciences (Bedford, MA, USA); CD3 (100203), F4/80 (123128), CCR2 (150608), CD44 (mouse and human 103007), CD74 (mouse (151005) and human (326811)) from BioLegend (St. Diego, CA, USA), MHCII (47-5321-80, Life Technologies/Thermo Fisher Scientific, NY, USA) and Tim4 (12-5866-82, Life Technologies/Thermo Fisher Scientific, NY, USA). Concentration of the different antibodies was determined by titration. In the meanwhile, compensation beads (UltraComp eBeads, Thermo Fisher) of the used primary antibodies were prepared. After staining, cells were washed with PBS and stored on ice until acquisition. For lipid raft analysis Vybrant Alexa Fluor 488 Lipid Raft Labeling Kit (Invitrogen) was employed. Acquisition was performed using BD FACSCanto TM II, BD LSR for analysis or Aria for cell sorting (BD Biosciences). Experiments were analyzed using FlowJo Software. Flow cytometer results in percentage were extrapolated to the total number of cells obtained from the cell counting.

**Whole mount staining**

The omentum was fixed for 1 hour in 1% PFA at room temperature and washed with PBS. Tissues were washed three times for 5 min with permeabilization buffer (PBS, 0.1% BSA and 0.2% Triton X-100) and treated with 0.5 mL blocking buffer (5% donkey serum diluted PBS-T) for 1 hour at room temperature. Primary antibodies, CD45 (BD 553076) and luciferase (ab185924), were diluted in PBS-T with 2% donkey serum. Next day, samples were washed three times with PBS- 0.3% Tween. Afterwards secondary antibodies (1:200) were incubated for 1 hour at room temperature, washed three times with PBS 0.3% Tween and incubated with DAPI (1:10.000) for 15 min. Next, samples were incubated with clearing solution (FUnGi: 60% glycerol (vol/vol), 2.5M fructose, 2.5M urea, 10mM Tris Base, 1.0mM EDTA) at 4 °C overnight.

Coverslips were mounted with FUnGi clearing solution and imaged with a confocal microscope (LSM 710, Carl Zeiss). All images were processed with ZENblue software (Carl Zeiss, Germany). Average mean intensities per image were counted with ImageJ software (NIH, Bethesda, MD, USA).

**Immunohistochemistry**

Paraffin-embedded sections (3 µm) were de-paraffinized and re-hydrated in xylene and step-wise reductions in alcohol concentrations. H&E staining was performed according to standard protocols. Cytokeratin DAB and CD11b: antigen retrieval was performed at pH9 with EDTA buffer. Primary antibodies CD11b (abcam, ab133357, 1:200), and Pan-cytokeratin (undiluted, ZUC001-125), diluted in blocking solution, were incubated at 4°C overnight. After washing, sections were incubated with secondary antibodies coupled with ZytoChem Plus (HRP) Polymer anti-mouse, (ZYTOMED, ZYT-ZUC050-006) and ZytoChem Plus (AP) Polymer anti-rabbit, (ZYTOMED, ZYT-ZUC031-006) diluted in antibody diluent (Cell Signaling) for one 30 min. at room temperature. Afterwards, slides were treated with DAB Substrate Chromogen, Zytomed, ZYT-DAB057 and AP Red Kit, Zytomed, ZUC001-125. Immunofluorescence staining: primary antibodies: CD31 (Abcam, ab28364, 1:50), VCAM (Vcam, ab134047, 1:200) and αSMA (Sigma-Aldrich, A5228, 1:200) were diluted in Animal free blocking solution (Cell Signaling). Fluorophore-conjugated secondary antibodies (Thermo Fisher Scientific) were diluted in SignailStain Antibody diluent (Cell Signaling) together with isolectin-B4 (Thermo Fisher Scientific, I32450). Images were obtained with slide scanner (Zeiss Axio Sacn.Z1, Carl Zeiss) and a confocal microscope (LSM 710, Carl Zeiss). All images were processed with ZENblue software (Carl Zeiss, Germany). Image quantification were proceeded with ImageJ software (NIH, Bethesda, MD, USA).

**Isolation of peripheral blood nuclear cells (PBMC) from buffy coats**

Human buffy coats were purchased from blood donation service DRK Mannheim, Germany. Peripheral blood nuclear cells (PBMC) were isolated by gradient centrifugation using

Biocoll density solution (L6715; Biochrom). Human buffy coat was diluted 1:1 with PBS and added to the Biocoll density solution. This mixture was centrifuged at 430g for 20 min at room temperature. After centrifugation, the white intermediate phase containing leukocytes was collected and washed with PBS. To perform a positive isolation of monocytes, CD14 MACS beads were used with the LS column (130-042-402; Milentyi Biotec). The isolation of $CD14^+$ monocytes was performed following the manufacture's protocol.

**Transwell assay**

Human ovarian cancer cells were seeded at 100,000 cells/ml in 500 µl RPMI medium without FCS for 48 hours. For the ECs monolayer, inserts were coated for 2 hours with 2 µg/ml fibronectin (1918-FN-02M; R&D Systems) in PBS. 50,000 human ECs (*RBPJ* knock-out or shRNA for *CXCL2* (pLKO.1; Sigma Aldrich) and respective controls) were seeded on top of the insert membrane for 48 hours. To analyze monocyte transmigration, 200,000 $CD14^+$ cells were stained with carboxyfluorescein succinimidyl ester (CSFE; ThermoFischer) and added onto the endothelial monolayer. The transwell plate was incubated for 2 hours. For the chemotaxis assay with the recombinant proteins, transmigration of 50,000 $CD14^+$ cells were analyzed towards 60 ng/mL CXCL2 (PeproTech GmbH) in RPMI medium without FCS for 30 min. After incubation, the remaining cell suspension in the upper well was aspirated and the transwell was cleaned with a cotton swab. The migrated cells were fixed with 4% PFA for 20 min at room temperature. Imaging of transwell was performed with Cell Observer (Carl Zeiss). From each transwell five evenly spaced field picture were taken using 20x objective and analysis was performed with Image J software.

**Enzyme-linked Immunosorbent Assay (ELISA)**

Protein expression of CXCL2 (MIP-2) was quantified using an enzyme-linked immunosorbent assay (ELISA; Abcam ab184862). Cell culture supernatant was collected after 24 hours and ELISA was performed following the manufacture's protocol.

**Bone marrow-derived macrophages (BMDMs) differentiation**

Mouse macrophages were derived from the bone marrow of wild-type C57BL/6 mice. Femurs and tibiae were flushed several times with DMEM and collected cells were centrifuged. Bone marrow cells were suspended in media and seeded on 10 cm petri dishes (Corning). To differentiate these cells into macrophages, 10 ng/ml M-CSF (PeproTech GmbH) were added to Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher) supplemented with 10% fetal calf serum (FCS) (Biochrom, UK). Differentiation occurred within seven days. Cells were afterwards stimulated with indicated amount of recombinant protein CXCL2 (250-15-20, PeproTech GmbH) in DMEM medium without FCS or conditioned medium of endothelial cells.

**Immunostaining**

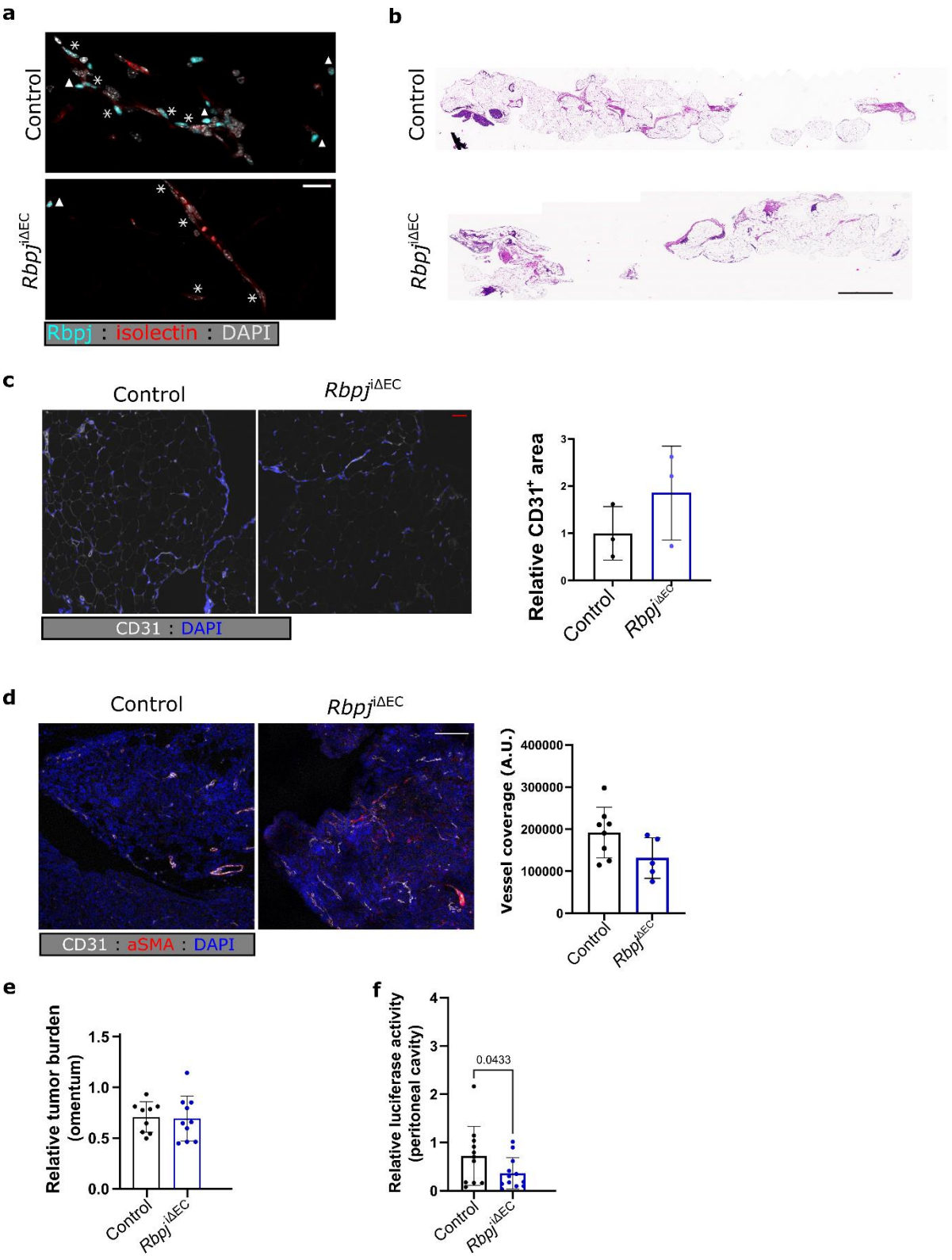
BMDMs were cultured in DMEM with 10% FCS (Biochrom, UK) and 250,000 cells/well were seeded into 24-well plates on top of coverslips. BMDMs were treated with 40 ng/mL CXCL2 (PeproTech GmbH) in DMEM without FCS for 72 hours. Cells were washed with PBS and fixed with 4% PFA for 10 min. Then, the coverslips were washed three times for 5 minutes with PBS, permeabilized with PBS with 0.1 % Triton X-100 for 10 minutes and blocked for 30 min in blocking buffer (PBS in 5% FCS with 0.1% Tween 20 and 100 mM glycine) for 1 hour at room temperature. The coverslips were incubated with antibody against CD44 (1:1000) (Abcam, ab124515) and CD45 (1:500) (BD 553076) overnight at 4°C. The coverslips were rinsed three times with blocking buffer and incubated with a secondary antibody coupled to Alexa Fluor-488 and Alexa Fluor-546 (1:200) for 1 hour. The coverslips were washed and incubated with a DAPI solution before they were washed again. Coverslips were mounted and imaged with a confocal microscope (LSM 710, Carl Zeiss). All images were processed with ZENblack software (Carl Zeiss, Germany).

**Table 1: Primers used for mouse genes**

| Gene Name | Forward Primer | Reverse Primer |
|---|---|---|
| ***Cph*** | ATGGTCAACCCCACCGTG | TTCTTGCTGTCTTTGGAACTTTGTC |
| ***Ccl1*** | CTGCTGCTTGAACACCTTGA | GGTGATTTTGAACCCACGTT |
| ***Ccl21*** | ACCCAAGGCAGTGATGGA | CAGGGTTTGCACATAGCTCA |
| ***Cxcl2*** | CCAACCACCAGGCTACAGG | GCGTCACACTCAAGCTCTG |
| ***Cxcl12*** | GTCCTCTTGCTGTCCAGCTC | TAATTTCGGGTCAATGCACA |
| ***Cd44*** | TCGATTTGAATGTAACCTGCCG | CAGTCCGGGAGATACTGTAGC |
| ***Mmp9*** | CTGGACAGCCAGACACTAAAG | CTCGCGGCAAGTCTTCAGAG |
| ***Arg1*** | CAGAAGAATGGAAGAGTCAG | CAGATATGCAGGGAGTCACC |

**Table 2: Primers used for human genes**

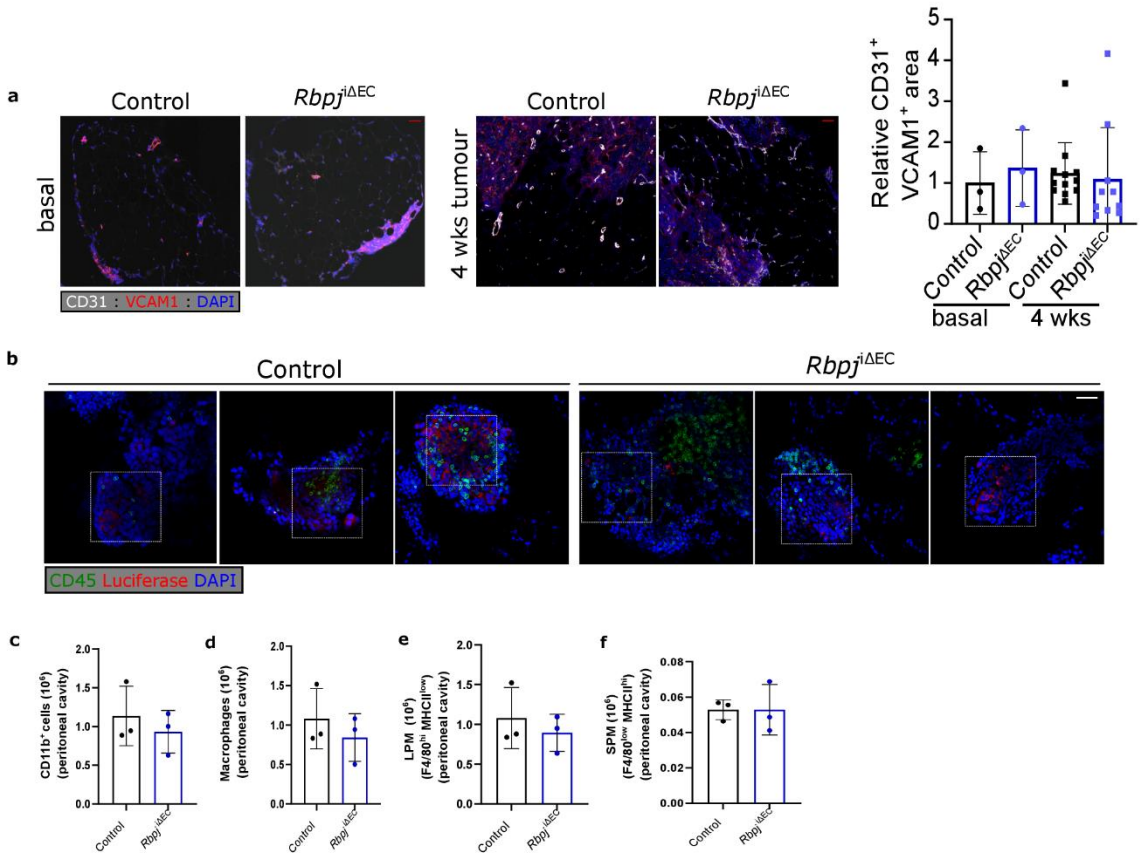
| Gene Name | Forward Primer | Reverse Primer |
|---|---|---|
| ***HPRT*** | TGTTGTAGGATATGCCCTTGACT | CTAAGCAGARGGCCACAGAAC |
| ***CCL1*** | CATTTGCGGAGCAAGAGATT | TGCCTCAGCATTTTTCTGTG |
| ***CCL2*** | CAGCCAGATGCAATCAATGCC | TGGAATCCTGAACCCACTTCT |
| ***CCL21*** | CCCAGCTATCCTGTTCTTGC | TCAGTCCTCTTGCAGCCTTT |
| ***CXCL2*** | GGCAGAAAGCTTGTCTCAACCC | CTCCTTCAGGAACAGCCACCAA |
| ***CXCL5*** | AGCTGCGTTGCGTTTGTTTAC | TGGCGAACACTTGCAGATTAC |
| ***CXCL8*** | AAGAAACCACCGGAAGGAAC | AAATTTGGGGTGGAAAGGTT |
| ***CXCL12*** | ATTCTCAACACTCCAAACTGTGC | ACTTTAGCTTCGGGTCAATGC |




**Supplementary Figure 1. Effects of loss of endothelial *Rbpj* in omental endothelium**

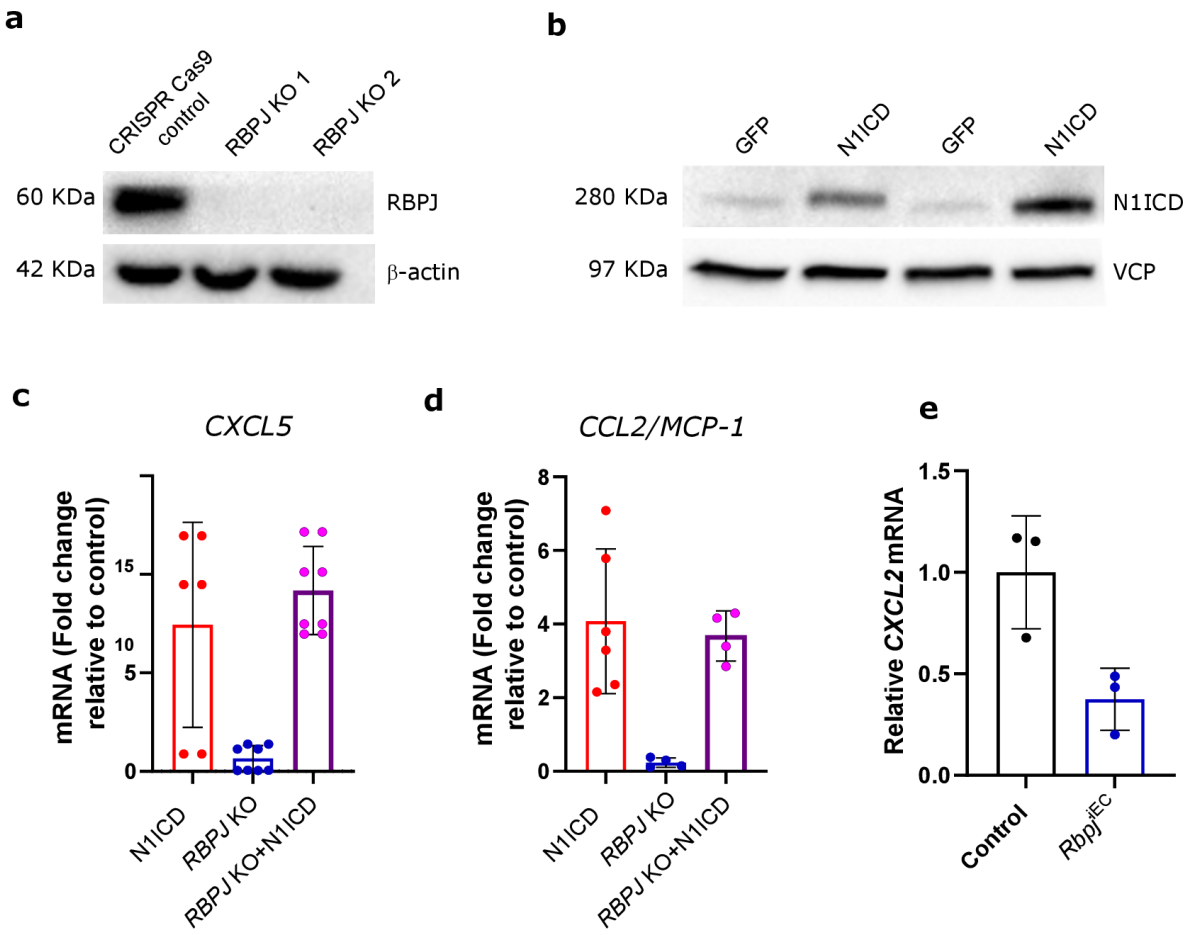
**a,** Representative high magnification microscopic images of omentum stained with RBPJ (turquoise), isolectin B4 (red) and DAPI (white) in omentum of non-tumor bearing Rbpj$^{i\Delta EC}$ and control mice. * indicates EC and Δ indicates non-EC. Scale bar, 50 μm. **b,** Representative

microscopic images of omentum stained with H&E from non-tumor bearing *Rbpj*$^{i\Delta EC}$ and control mice. Scale bar, 1mm. **c**, Representative images of immunohistochemistry staining for CD31 (white) and DAPI (blue) in omentum of non-tumor bearing *Rbpj*$^{i\Delta EC}$ and control mice. Scale bar, 50 µm. Bar graphs show mean±SD; two-tailed, unpaired Mann-Whitney U-test. **d,** Representative images of immunohistochemistry staining for CD31 (white), αSMA (red) and DAPI (blue) in omentum of *Rbpj*$^{i\Delta EC}$ and control mice after four weeks of ID8 tumor growth. Scale bar, 100 µm. Quantification of vessel density ($CD31^+$) and vessel coverage ($\alpha SMA^+/CD31^+$) n≥5. Bar graphs show mean±SD; two-tailed, unpaired Mann-Whitney U-test. **e,** Tumor burden quantification from whole omentum at six weeks after tumor injection of control (n=5 and 6) and *Rbpj*$^{i\Delta EC}$ mice (n=9 and 10). Bar graphs show mean±SD; two-tailed, Welch's corrected t-test **f**, Luciferase activity in peritoneal cavity of tumor-bearing *Rbpj*$^{i\Delta EC}$ compared to control mice six weeks after tumor injection. Quantification of luciferase levels in control (n=11) and *Rbpj*$^{i\Delta EC}$ (n=12). Bar graphs show mean±SD; one-tailed, Welch's corrected t-test.



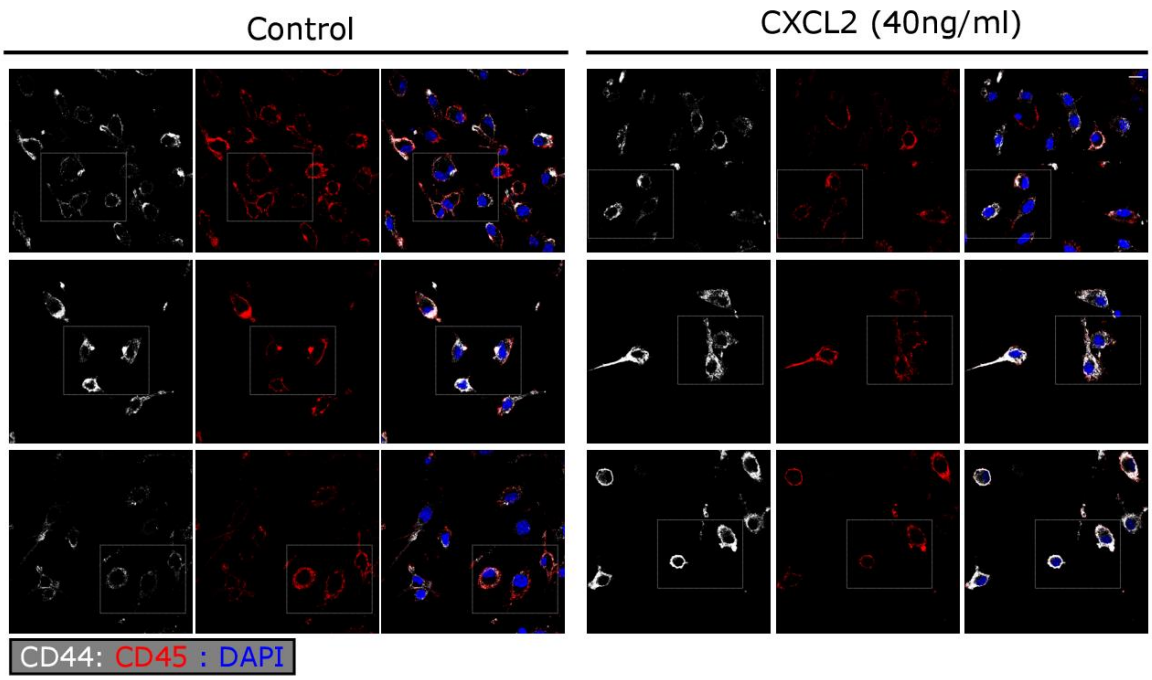

**Supplementary Figure 2. Effects of loss of endothelial *Rbpj* on myeloid cell infiltration under physiological conditions**

**a,** Representative images of immunohistochemistry staining for CD31 (white), VCAM1 (red) and DAPI (blue) in omentum of non-tumor bearing and four weeks after tumor growth from *Rbpj*$^{i\Delta EC}$ and control mice. Scale bar, 50 µm. Quantification of n≥3. Bar graphs show mean±SD; two-tailed, unpaired Mann-Whitney U-test. **b**, Full representative images of whole mount staining of tumor nodules of the omentum for luciferase (red), CD45 (green) and DAPI (blue) four weeks after tumor injection in *Rbpj*$^{i\Delta EC}$ and control mice. Scale bar, 20 µm.**c**, Myeloid cells (CD45$^{+}$, CD11b$^{high}$) and **d,** Macrophages (CD45$^{+}$, CD11b$^{high}$, F4/80$^{+}$) relative to alive cells. n=3. Bar graphs show mean ±SD; two-tailed, unpaired Mann-Whitney U-test. **e** and **f,** macrophage subpopulations characterization by F4/80 and MHCII expression into LPM and SPM in non-tumor bearing *Rbpj*$^{i\Delta EC}$ mice compared to controls and their quantification. n=3. Bar graphs show mean ±SD; two-tailed, unpaired Mann-Whitney U-test.



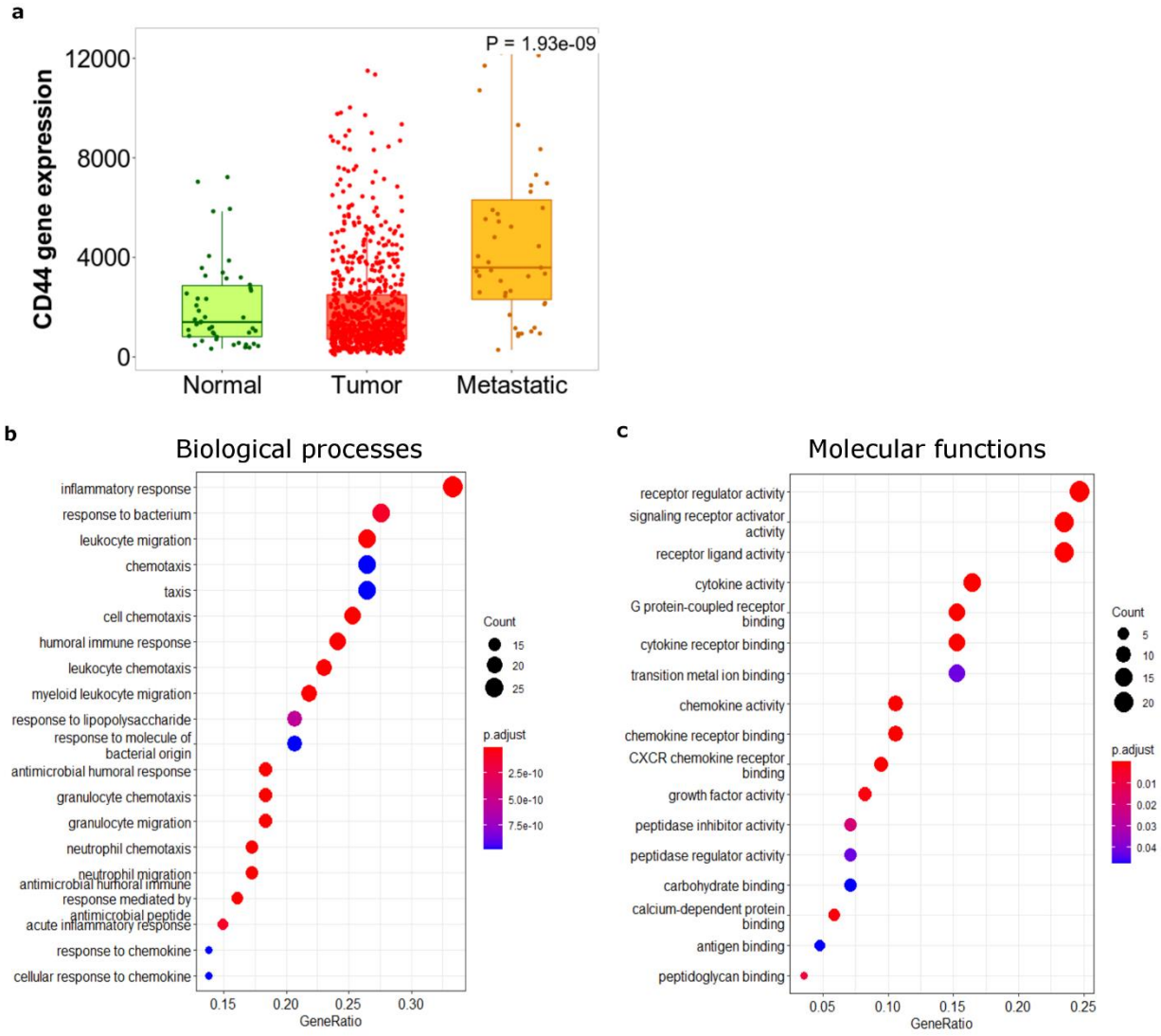

**Supplementary Figure 3. RBPJ-independent Notch1 regulation of cytokines in endothelial cells**

**a,** western blot of RBPJ and β-actin in two different CRISPR-Cas9 mediated knock-out (KO) HUVECs and their control. **b**, western blot of Notch1 and VCP in HUVECs infected with N1ICD and GFP, as control. Quantification of mRNA expression of **c**, *CXCL5* and **d**, *CCL2* upon NICD overexpression, RBPJ KO of and their combination in HUVEC. n≥6. Bar graphs show mean ±SD; two-tailed, unpaired Mann-Whitney U-test. **e**, Quantification of *CXCL2* mRNA expression in isolated EC from lungs of $Rbpj^{i\Delta EC}$ and control mice. n=3. Bar graphs show mean±SD; two-tailed, unpaired Mann-Whitney U-test.



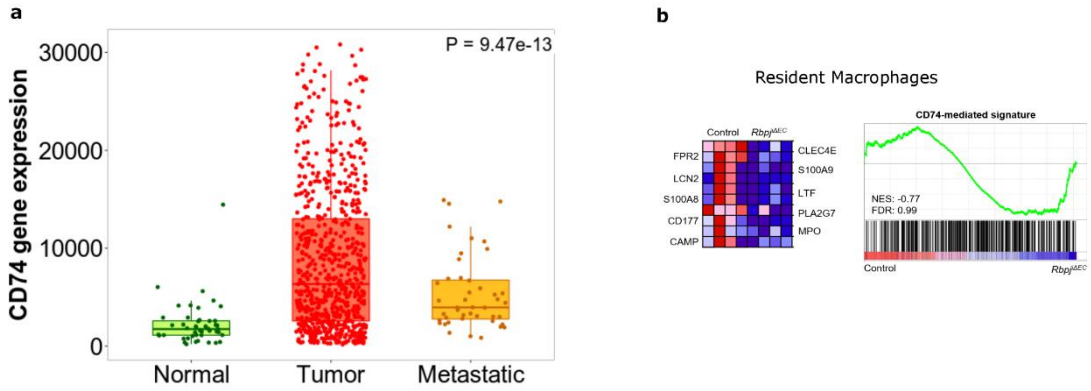

**Supplementary Figure 4. Essential role of endothelial *Rbpj* in regulating CXCL2 mediated expression of CD44 on macrophages**

Full representative images of BMDMs stained with CD44 (red), CD45 (green) and DAPI (blue) of control and stimulated with Cxcl2 (40 ng/mL).




**Supplementary Figure 5. Gene ontology analysis of ovarian cancer patients divided in CXCL2 expression high and low.**

**a**, Differential expression among normal, primary and metastatic tumor of *CD44* in samples coming from ovarian cancer patients and healthy donors. **b**, **c**, Gene ontology (GO)-term analysis, biological processes (**b**) and molecular function (**c**) of $CXCL2^{high}$ patients using R Studio software (R Core Team (2017)); n=97.




**Supplementary Figure 6. No changes in CD74-mediated gene signature for resident peritoneal macrophages (CCR2$^-$).**

**a**, Differential expression among normal, primary and metastatic tumor of *CD74* in samples coming from ovarian cancer patients and healthy donors. **b**, GSEA of resident macrophages (CD45$^+$; CD11b$^+$; F4/80$^+$; CCR2$^-$) from *Rbpj*$^{i\Delta EC}$ and control mice compared with CD74-mediated gene signature with 10 most differentially regulated gene extracted from TAM signature of ID8 tumor growth, n=4.