

# **Supplementary Information**

## **“Secreted Factors from Colorectal and Prostate Cancer Cells Skew the Immune Response in Opposite Directions”**

**Marie Lundholm<sup>1</sup>, Christina Hägglöf<sup>1</sup>, Maria L. Wikberg<sup>1</sup>, Pär Stattin<sup>2</sup>, Lars Egevad<sup>3</sup>, Anders Bergh<sup>1</sup>, Pernilla Wikström<sup>1</sup>, Richard Palmqvist<sup>1</sup>, and Sofia Edin<sup>1,\*</sup>**

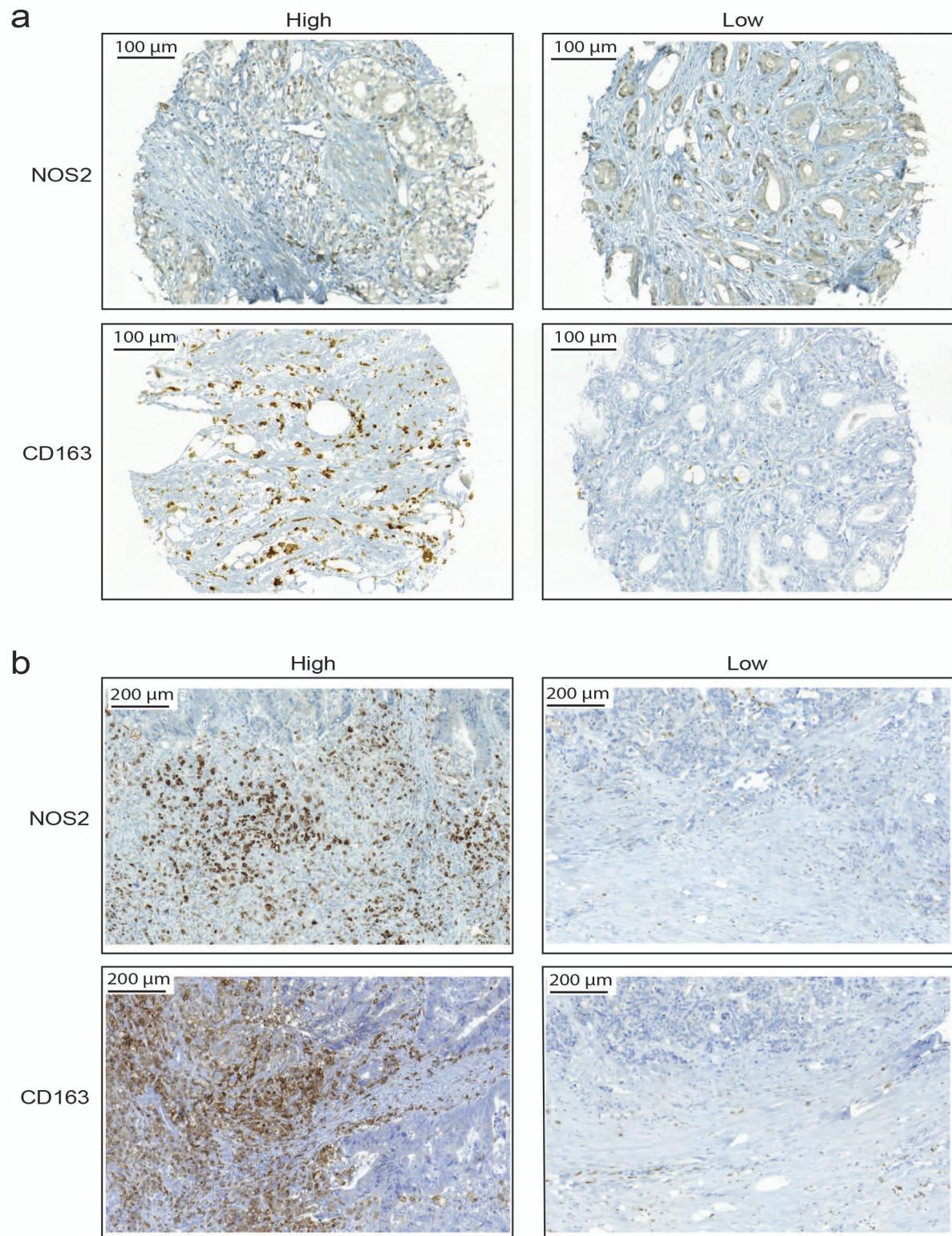
<sup>1</sup>Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden.

<sup>2</sup>Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden.

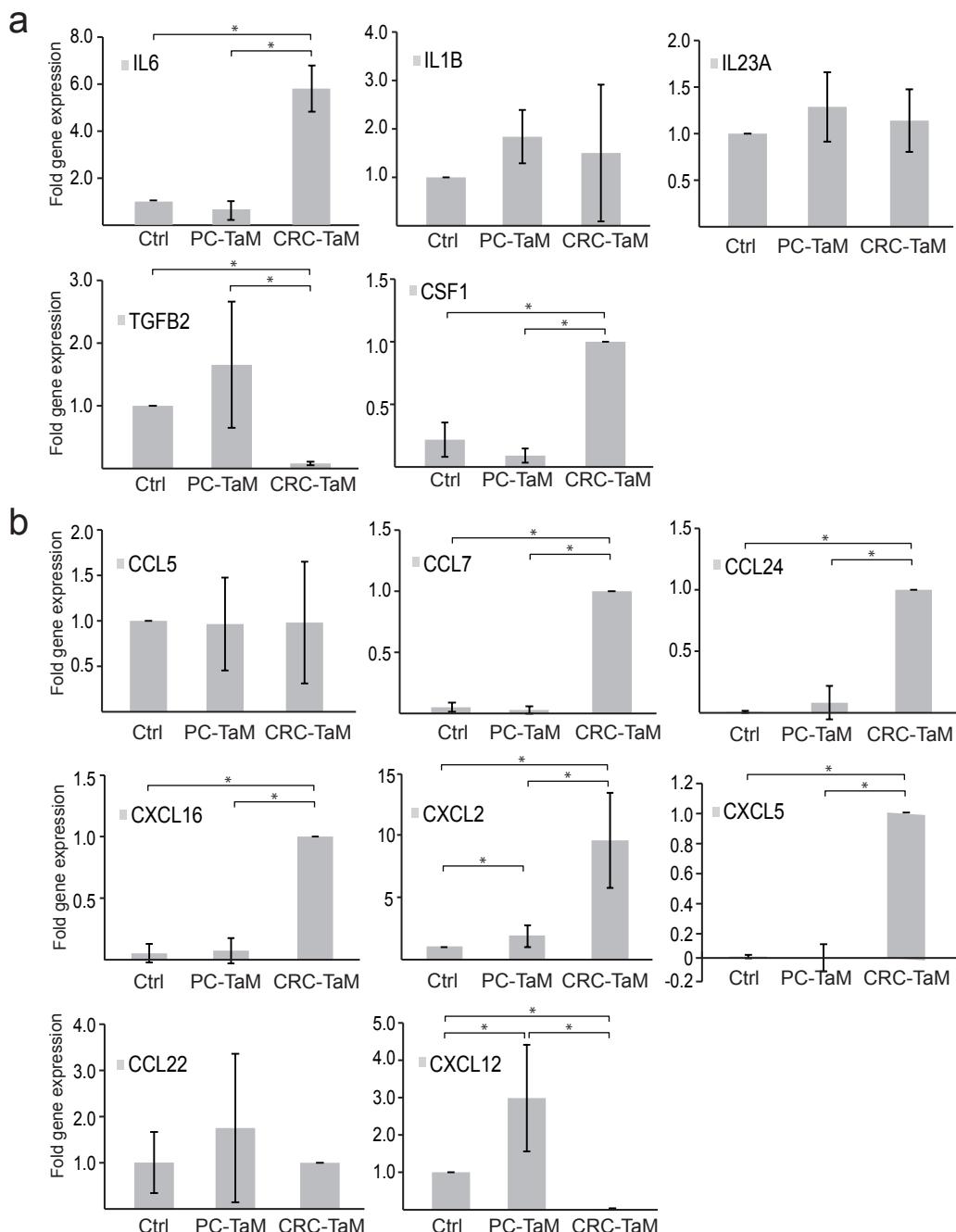
<sup>3</sup>Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden.

\*sofia.edin@umu.se

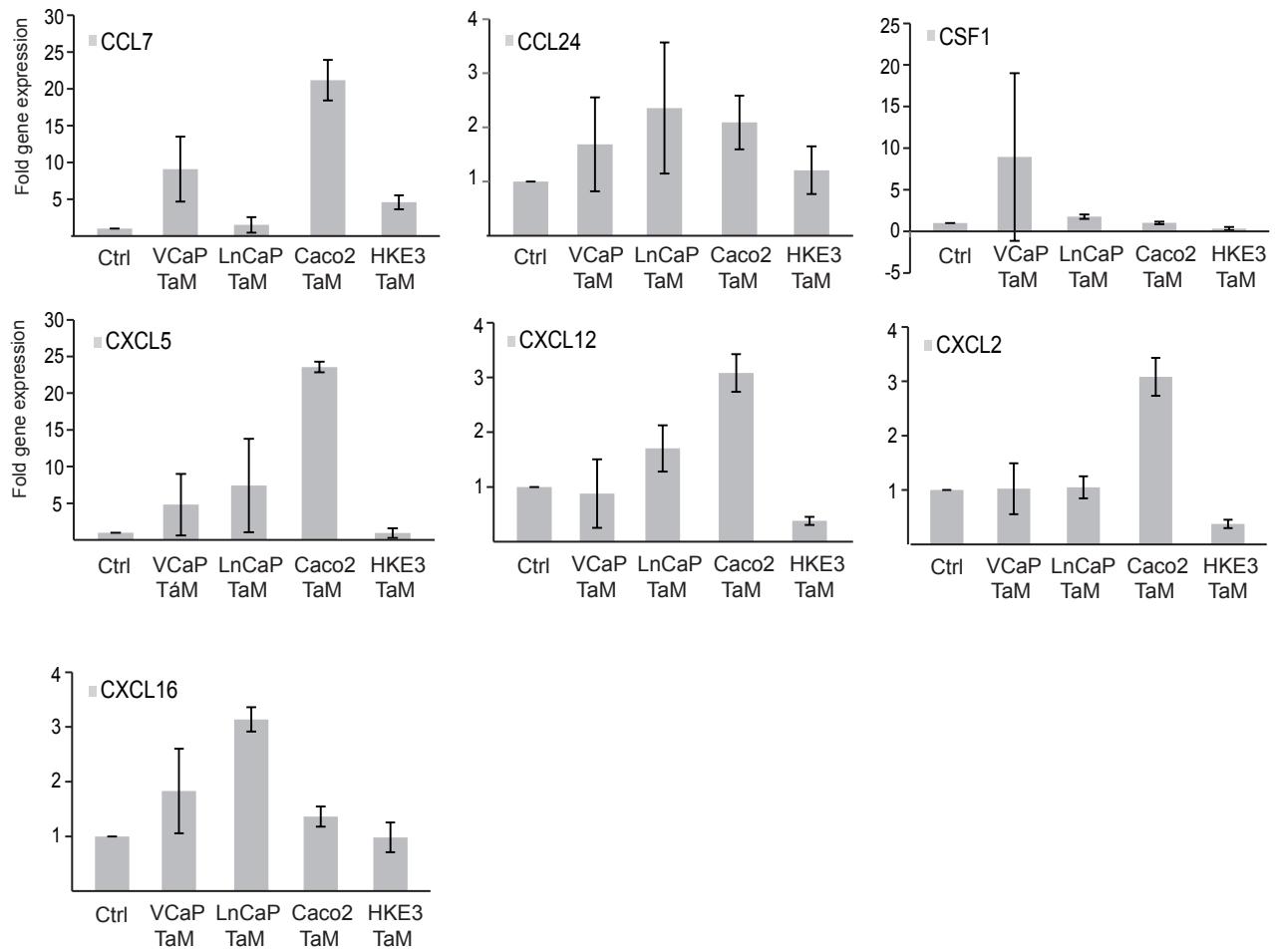
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



## **Supplementary Figure Legends**

**Figure S1.** Representative images of immunohistological staining of NOS2 and CD163. Shown are tumor specimens with high and low infiltration of immunoreactive macrophages in PC (a) and CRC (b).

**Figure S2.** Expression of cytokine and chemokine genes by TaMs. Gene expression of differentiated macrophages (Ctrl) or PC-TaMs or CRC-TaMs was analyzed by RT-PCR. Shown is the fold gene expression from three or more independent experiments  $\pm$  SD, with Ctrl macrophages or CRC-TaMs set as 1. Significant differences are indicated by \* ( $P \leq 0.05$ ). (A) Expression of proinflammatory and immunosuppressive cytokines. (B) Expression of chemokines associated with Th1/CTL and Th2/Treg recruitment and polarization.

**Figure S3.** Expression of cytokines and chemokines in TAMs induced by conditioned medium from PC cells VCaP (VCaP-TaM) and LnCAP (LnCaP-TaM), or CRC cells Caco2 (Caco2-TaM) and HKE3 (HKE3-TaM). Gene expression was analyzed by RT-PCR. Shown is the fold gene expression  $\pm$  SD with Ctrl macrophages set as 1.

**Supplementary Table S1.** Cytokine and chemokine expression in differentiated macrophages (Ctrl), PC-TaMs and CRC-TaMs.

Gene symbol	Ctrl	PC-TaM	CRC-TaM
<i>ADIPOQ</i>	1,62E-05	2,26E-05	2,00E-05
<i>BMP2</i>	4,18E-04	8,24E-05	2,00E-05
<i>BMP4</i>	4,15E-05	2,26E-05	2,00E-05
<i>BMP6</i>	1,62E-05	2,26E-05	2,28E-04
<i>BMP7</i>	1,62E-05	2,26E-05	2,00E-05
<i>C5</i>	3,51E-03	3,64E-03	6,83E-03
<i>CCL1</i>	1,62E-05	1,22E-04	7,57E-04
<i>CCL11</i>	6,29E-05	2,33E-04	3,13E-05
<i>CCL13</i>	6,37E-04	7,32E-04	2,30E+00
<i>CCL17</i>	1,62E-05	2,26E-05	1,02E-04
<i>CCL18</i>	6,54E-04	1,74E-03	5,92E-02
<i>CCL19</i>	1,62E-05	2,26E-05	6,48E-05
<i>CCL2</i>	1,52E+0	1,57E+00	2,09E+00
<i>CCL20</i>	1,62E-05	9,94E-05	2,30E-04
<i>CCL21</i>	1,62E-05	2,26E-05	4,97E-05
<i>CCL22</i>	1,71E-04	1,28E-03	4,69E-04
<i>CCL24</i>	1,43E-04	1,35E-04	1,63E-01
<i>CCL3</i>	3,79E-03	1,71E-02	7,73E-03
<i>CCL5</i>	1,18E-03	3,40E-03	4,86E-03
<i>CCL7</i>	1,80E-04	6,93E-04	3,68E-01
<i>CCL8</i>	1,62E-05	2,26E-05	8,04E-05
<i>CD40LG</i>	2,10E-04	2,40E-04	3,19E-04
<i>CNTF</i>	3,03E-03	1,12E-03	2,15E-03
<i>CSF1</i>	2,39E-04	7,01E-04	6,34E-03
<i>CSF2</i>	4,96E-05	2,68E-04	6,67E-05
<i>CSF3</i>	3,79E-04	4,24E-04	4,01E-04
<i>CX3CL1</i>	5,77E-05	1,20E-04	2,00E-05
<i>CXCL1</i>	1,18E-02	2,93E-02	5,69E-02
<i>CXCL10</i>	1,11E-03	4,06E-04	9,40E-04
<i>CXCL11</i>	1,62E-05	2,26E-05	2,00E-05
<i>CXCL12</i>	1,92E-04	1,00E-03	2,00E-05
<i>CXCL13</i>	4,00E-05	2,26E-05	1,13E-04
<i>CXCL16</i>	1,07E-02	3,36E-02	5,87E-02
<i>CXCL2</i>	2,23E-02	8,57E-02	2,31E-01
<i>CXCL5</i>	5,40E-04	6,18E-03	2,95E+00
<i>CXCL9</i>	3,27E-05	3,53E-05	5,58E-05
<i>FASLG</i>	1,62E-05	2,26E-05	2,00E-05
<i>GPI</i>	1,06E-01	1,38E-01	1,62E-01
<i>IFNA2</i>	1,62E-05	2,26E-05	2,00E-05
<i>IFNG</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL10</i>	6,26E-03	7,76E-03	4,54E-03
<i>IL11</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL12A</i>	1,62E-05	1,07E-04	2,00E-05
<i>IL12B</i>	1,62E-05	2,26E-05	9,61E-05
<i>IL13</i>	1,02E-04	1,80E-04	1,94E-04

<i>IL15</i>	1,28E-03	1,20E-03	3,46E-03
<i>IL16</i>	1,84E-02	2,88E-02	2,07E-02
<i>IL17A</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL17F</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL18</i>	9,35E-02	7,55E-02	7,84E-02
<i>IL1A</i>	1,39E-03	3,09E-04	1,73E-03
<i>IL1B</i>	2,11E-03	2,49E-03	1,51E-02
<i>IL1RN</i>	6,22E-03	1,16E-02	1,98E-01
<i>IL2</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL21</i>	4,35E-05	1,12E-04	9,12E-05
<i>IL22</i>	1,09E-04	1,59E-04	2,19E-04
<i>IL23A</i>	5,73E-04	1,99E-04	5,23E-04
<i>IL24</i>	2,07E-05	1,24E-04	1,05E-04
<i>IL27</i>	1,13E-04	1,13E-04	4,14E-04
<i>IL3</i>	1,62E-05	2,26E-05	2,00E-05
<i>IL4</i>	5,82E-05	2,26E-05	2,00E-05
<i>IL5</i>	1,62E-05	2,26E-05	1,47E-04
<i>IL6</i>	1,47E-03	3,72E-04	9,96E-03
<i>IL7</i>	1,03E-03	6,60E-04	4,30E-04
<i>IL8</i>	7,16E-03	9,99E-02	5,29E-01
<i>IL9</i>	2,21E-05	4,11E-05	1,73E-04
<i>LIF</i>	1,55E-04	1,10E-04	2,39E-04
<i>LTA</i>	1,62E-05	2,31E-05	2,00E-05
<i>LTB</i>	5,30E-04	4,96E-04	3,93E-03
<i>MIF</i>	1,71E-01	1,69E-01	3,29E-01
<i>MSTN</i>	2,20E-05	2,95E-05	2,00E-05
<i>NODAL</i>	1,34E-04	8,25E-05	2,38E-04
<i>OSM</i>	4,54E-03	1,98E-03	2,59E-03
<i>PPBP</i>	6,49E-04	2,43E-03	1,75E+01
<i>SPP1</i>	7,30E-01	3,15E-01	1,13E+00
<i>TGFB2</i>	4,22E-03	3,35E-03	5,13E-04
<i>THPO</i>	1,62E-05	2,26E-05	2,00E-05
<i>TNF</i>	9,60E-03	7,12E-03	1,39E-02
<i>TNFRSF11</i>	1,62E-05	2,26E-05	2,14E-05
<i>TNFSF10</i>	1,15E-02	1,25E-02	6,32E-02
<i>TNFSF11</i>	1,56E-04	3,62E-04	9,55E-05
<i>TNFSF13B</i>	3,63E-02	2,74E-02	3,87E-02
<i>VEGFA</i>	4,27E-03	6,79E-03	1,75E-02
<i>XCL1</i>	1,62E-05	2,26E-05	2,00E-05

Normalized expression of cytokine and chemokine genes, according to the  $2^{-\Delta Ct}$  ( $Ct(GOI) - Ave Ct(HKG)$ ) data analysis method. GOI; Gene of interest, HKG; House keeping gene.