



*Supporting Information*

# Differential Modulation of Human M1 and M2 Macrophage Activity by ICOS-Mediated ICOSL Triggering

Casimiro Luca Gigliotti <sup>1,2,†</sup>, Chiara Dianzani <sup>3,†</sup>, Ian Stoppa <sup>1</sup>, Chiara Monge <sup>3</sup>, Salvatore Sutti <sup>1</sup>, Daniele Sblattero <sup>4</sup>, Chiara Puricelli <sup>1,5</sup>, Roberta Rolla <sup>1,5</sup>, Umberto Dianzani <sup>1,5,\*</sup> and Elena Boggio <sup>1,2,‡</sup>

<sup>1</sup> Department of Health Sciences, Università del Piemonte Orientale, 28100 Novara, Italy

<sup>2</sup> NOVAICOS s.r.l.s, Via Amico Canobio 4/6, 28100 Novara, Italy

<sup>3</sup> Department of Scienza e Tecnologia del Farmaco, University of Turin, 10125 Turin, Italy

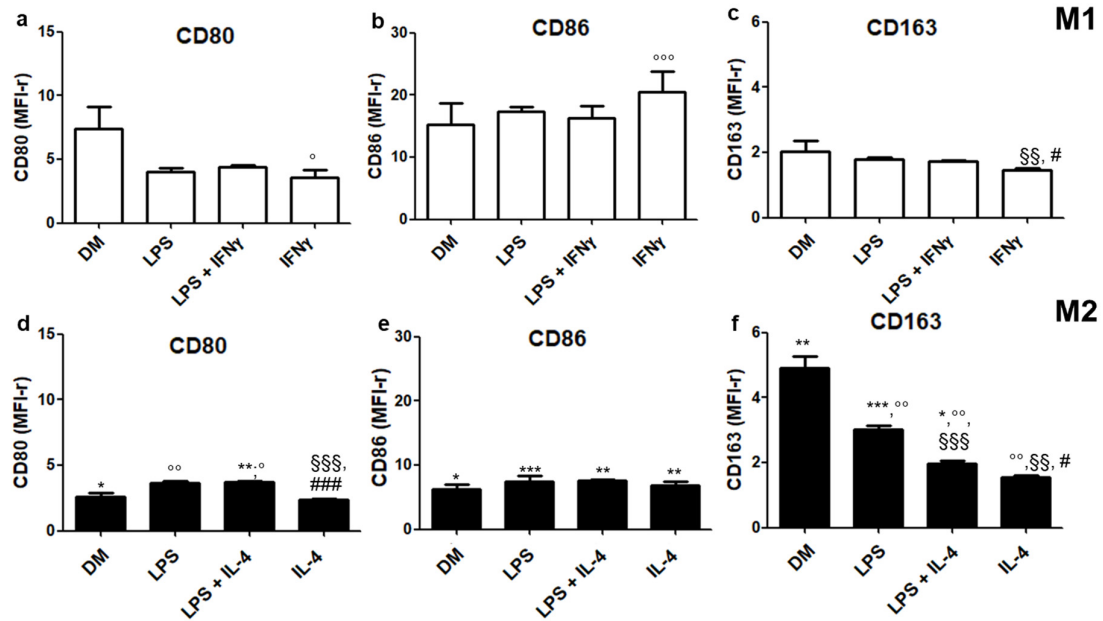
<sup>4</sup> Department of Life Sciences, University of Trieste, 34127 Trieste, Italy

<sup>5</sup> Clinical Biochemistry Laboratory, Maggiore della Carità University Hospital, 28100 Novara, Italy

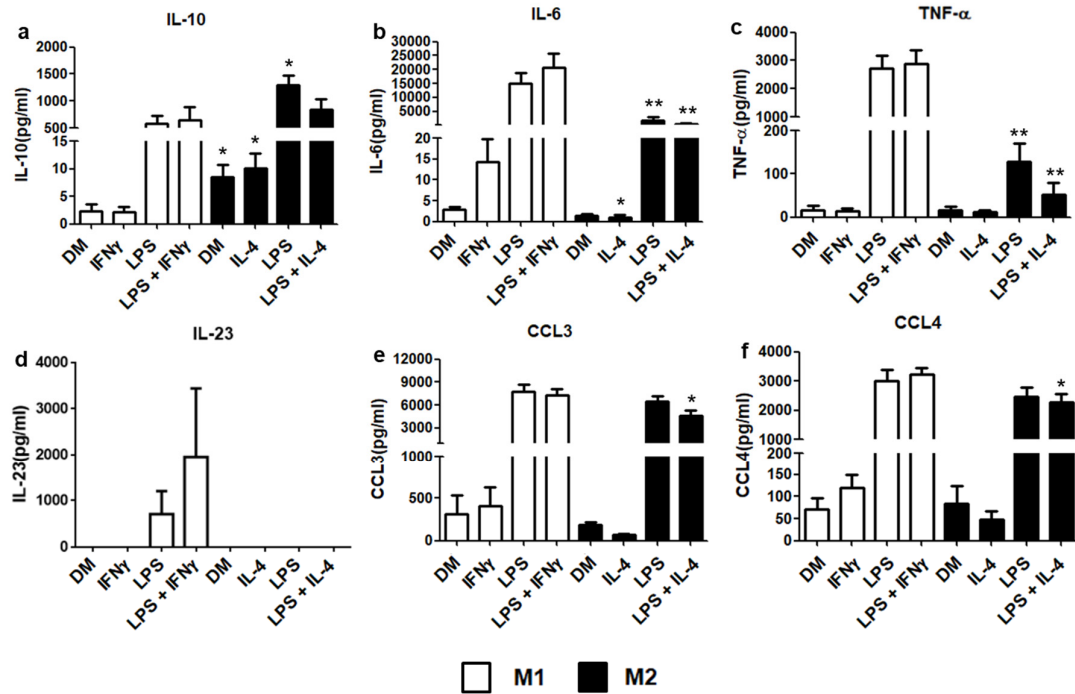
\* Correspondence: [umberto.dianzani@med.uniupo.it](mailto:umberto.dianzani@med.uniupo.it); Tel.: +39-032-1373-3487

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.



**Figure S1.** CD80, CD86, and CD163 expression on M1 and M2 cells. Monocytes were cultured in differentiation medium (DM), (GM-CSF for M1 polarization or M-CSF for M2 polarization). CD80 (a–d), CD86 (b–e), and CD163 (c–f) expression was evaluated after 6 days (d) (T6) of culture in the presence of GM-CSF or M-CSF alone or after an additional 48 hours (h) (T6 + 2) in the presence of LPS, IFN $\gamma$ , or LPS+IFN $\gamma$  (M1) or LPS, IL-4, or LPS+IL-4 (M2) by flow cytometry. Data represent the mean and SEM expressed as mean fluorescence intensity ratio (MFI-R) of results from five independent experiments. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$  versus the corresponding M1; SS  $p < 0.01$ , SSS  $p < 0.0001$  vs LPS or °  $p < 0.05$ , °°  $p < 0.01$ , °°°  $p < 0.001$  vs. DM; #  $p < 0.05$ , ###  $p < 0.0001$  vs. LPS+ IFN $\gamma$  or LPS+IL-4.



**Figure S2.** Secretion of cytokines/chemokines by M1 (white bars) and M2 (black bars) macrophages activated with different stimuli. Culture supernatants were harvested and examined for IL-10 (a), IL-6 (b), TNF- $\alpha$  (c), IL-23 (d), CCL3 (e), and CCL4 (f) production by ELISA. Results are expressed as

pg/ml and represent the mean  $\pm$  SEM of 5–6 experiments. Comparison of M1 with corresponding M2 cells: \*  $p < 0.05$ ; \*\*  $p < 0.01$  unpaired T-test. DM: Differentiation media.

**Table S1.** Migration of M1 and M2 macrophages activated with different stimuli in response to either CCL7 or OPN. Results are expressed as the number of migrating cells and are the mean $\pm$ SEM of the results from 3–22 experiments. Comparison between M1 and corresponding M2 cells: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ , unpaired T test.

M1 cells			
Chemoattractant	IFN $\gamma$	LPS	LPS + IFN $\gamma$
CCL7	122 $\pm$ 17	123 $\pm$ 21	146 $\pm$ 27
OPN	118 $\pm$ 26	130 $\pm$ 26	182 $\pm$ 57
M2 cells			
Chemoattractant	IL-4	LPS	LPS + IL-4
CCL7	163 $\pm$ 25***	101 $\pm$ 26**	168 $\pm$ 44
OPN	163 $\pm$ 43 (p=0.06)	193 $\pm$ 42**	142 $\pm$ 48