

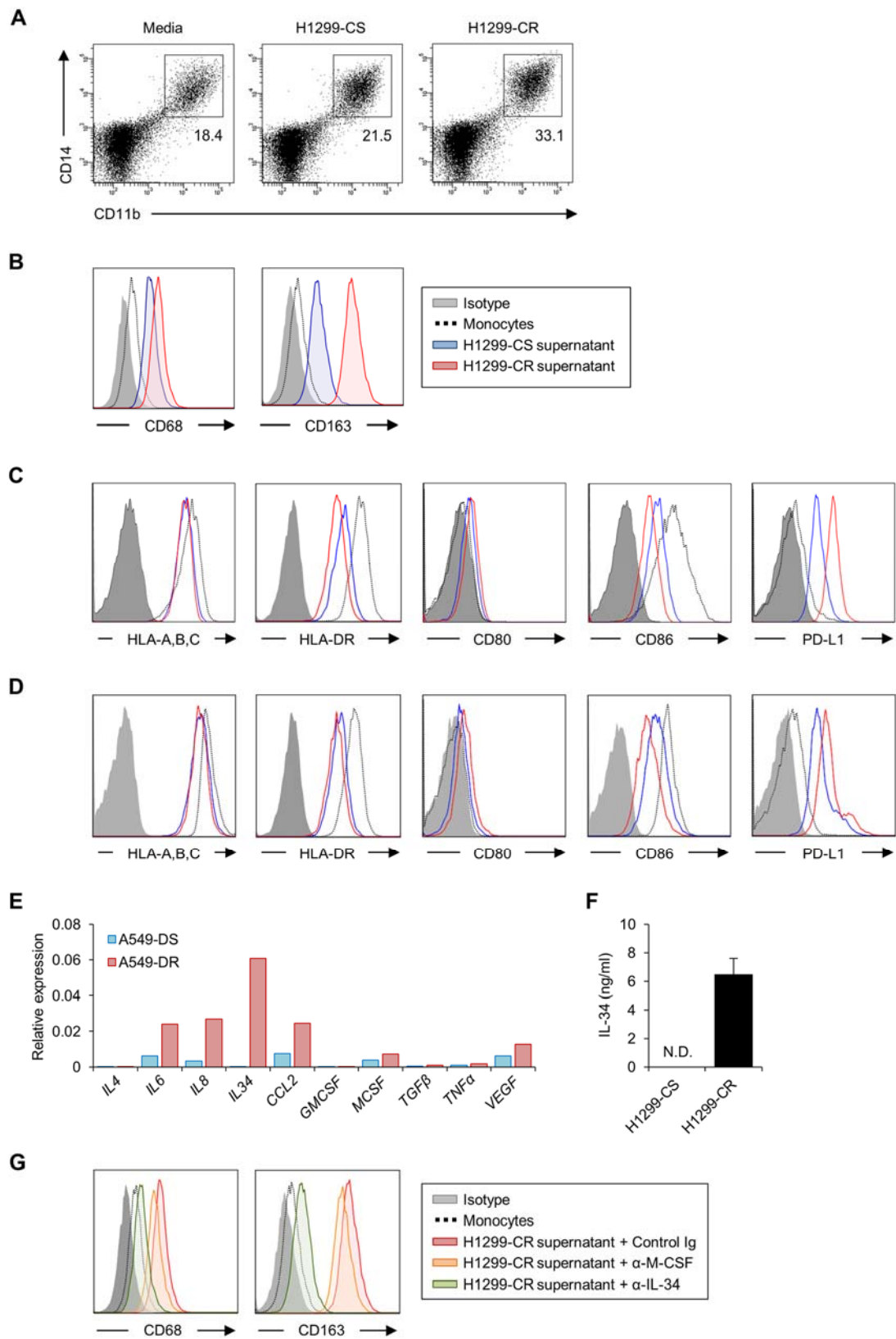


HOKKAIDO UNIVERSITY

Title	Chemotherapy-Induced IL34 Enhances Immunosuppression by Tumor-Associated Macrophages and Mediates Survival of Chemoresistant Lung Cancer Cells
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Supplementary figure 2



IL-34 in the supernatant of chemoresistant lung cancer cells mediates its effects on monocytes differentiation. A, frequencies of CD14⁺CD11b⁺ fraction in total peripheral blood leukocytes (PBLs) treated for 7 days with media or supernatants of chemosensitive (H1299-CS) or chemoresistant (H1299-CR) cells as evaluated by flow cytometry. B, CD68 or CD163 expression in purified CD14⁺ monocytes cultured in media or in the presence of H1299-CS or H1299-CR supernatants for 7 days as evaluated by flow cytometry. C and D, flow cytometry analysis for HLA-A,B,C, HLA-DR, CD80, CD86 and PD-L1 in purified CD14⁺ monocytes cultured in the presence of A549 (C) or H1299 (D) supernatants for 7 days. Gray histogram: isotype, black dotted line: monocytes, blue line: supernatant of chemosensitive cancer cells, red line: supernatant of chemoresistant cancer cells. E, comparison of cytokines and chemokines expression between A549-DS and A549-DR cells as evaluated by RT-qPCR. F, ELISA measurement of IL-34 in the supernatants of chemosensitive (H1299-CS) or chemoresistant (H1299-CR) cells as measured in undiluted 72 hours culture. G, purified CD14⁺ monocytes were cultured in media or in the presence of H1299-CR supernatants pretreated with α -M-CSF, α -IL-34 or control Ig (rabbit polyclonal and mouse IgG1 isotype control) for 7 days. The expression of CD68 or CD163 was evaluated on day 7 by flow cytometry. Large cells were gated based on FSC/SSC. N.D. Not detected.