Supplementary Materials and Methods

Binding of anti-ILT3 to human primary cells and SK-MEL-5–educated M-MDSCs

For the assessment of binding of anti-ILT3 to human primary cells, PBMCs (2×10^5) or whole blood (100 µL) from healthy human donors or $CD33^+$ M-MDSC (2×10^5), cells were stained with serially diluted concentrations of Alexa Fluor 647 labeled anti-ILT3 antibody c52B8 or isotype control hIgG4 (ranging from 1 µg/mL to 0.15 ng/mL) and a cocktail of antibodies (0.1 µg each of CD45, CD3, CD19, 0.05µg each of CD14, CD66b and 0.04 µg of CD33) (**Supplementary Table S1**) at room temperature for 1 hour. After the incubation, FACS lysing solution (BD Biosciences) was added to whole blood samples only, and the cells were incubated at room temperature for 10 minutes. The cells were washed in cell-staining buffer, resuspended in Cytofix buffer, and incubated at 37°C for 15 minutes. After the incubation, the cells were processed and analyzed as described previously for phenotyping of human M-MDSCs. Binding signals were calculated as MFI for each concentration of drug.

Supplementary Figure Legends

Supplementary Figure S1. SK-MEL-5-educated monocytes have M-MDSC like properties compared to normal myeloid counterparts from PBMCs cultured in medium alone. (**A-B**) A representative dot plot from flow cytometry analysis showing CD14 and HLA-DR expression in $CD45^+CD33^+$ PBMCs cultured in medium alone and SK-MEL-5-educated M-MDSCs. (**C**) A representative histogram showing cell surface staining of SK-MEL-5-educated M-MDSCs and PBMCs cultured in medium alone by staining with anti-CD33, CD14, CD11b, HLA-DR, CD66b and ILT3 antibodies. (**D**) A representative FACS result of cell surface staining of cytokine-induced M-MDSCs and PBMCs cultured in medium alone by staining with anti-CD33, CD14, CD11b, HLA-DR, CD66b and ILT3 antibodies. (**E**) A representative histogram depicting cell surface staining of monocytes cultured in the presence of M-CSF and IL4 and SK-MEL-5-educated M-MDSCs by staining with HLA-DR.

Supplementary Figure S2. ILT3 is highly expressed on $CD45^+CD33^+CD14^+$ myeloid cells in SK-MEL-5 tumor-infiltrating lymphocytes. (**A-B**) A representative image of the flow cytometry analysis for the myeloid cell compartment. SK-MEL-5 tumor cells grown subcutaneously in humanized mice, pooled tumor samples from 5 mice euthanized at day 11 after implantation. The total $CD45^+$ cell population is included in the plots.

Supplementary Figure S3. Single-cell RNAseq (scRNAseq) of human immune cells in non-small cell lung cancer (NSCLC) public dataset (Zilionis et al, 2019). (**A**) Two-dimensional visualization (UMAP plots) of immune cell transcriptomes from patient blood (n=6) and tumor samples (n=7). Cells colored by major cell types (t, tumor; b, blood). LILRB4 (ILT3) and LILRB2 (ILT4) expression are shown in (**B**) and (**C**), respectively. LILRB4 (ILT3) expression was limited mostly to the tMoMacDC, bMonocytes, and pDC cells, while LILRB2 (ILT4)

expression was mostly limited to neutrophils, tMoMacDC, and bMonocytes. The dataset was processed by, and plots generated using, BioTuring Browser, ver2.2.20 (BioTuring Inc., San Diego, CA, USA).

Supplementary Figure S4. Association of LILRB4 expression with deconvoluted cell fractions for 4 human tumor types: head & neck, kidney/clear cell, lung adenocarcinoma and pancreatic cancer in The Cancer Genome Atlas (TCGA) database. Deconvolution analysis was performed separately for each tumor type. In each tumor type LILRB4 expression showed the strongest association with $CD14^+CD33^+CD11b^+$ monocytic myeloid cells.

Supplementary Figure S5. Suppression of autologous $CD8^+$ T cell proliferation by cytokine-induced M-MDSCs (T cell:MDSC ratio: 8:1, 4:1, 2:1, 1:1). (**A** and **B**) $CD8^+$ T cells were stimulated with anti-CD3/CD28 beads and incubated with or without MDSCs for 3 days. T cell proliferation was analyzed using 3H-thymidine uptake assay. The results are expressed as average ± standard deviation from 2 technical replicates (n=2 PBMC donors), and one-way ANOVA followed by Dunnett's correction was used to calculate *P*-value, ***$P < 0.0005$; ****$P < 0.0001$; n.s., not statistically significant.

Supplementary Figure S6. $CD33^+$ cells from PBMCs cultured in medium alone are not suppressive (T cell:MDSC ratio: 4:1). (**A-B**) $CD8^+$ T cells were stimulated with anti-CD3/CD28 antibody and incubated with SK-MEL-5-educated M-MDSCs or $CD33^+$ myeloid cells from PBMCs cultured in medium alone for 3 days and IFN-γ concentration was determined in supernatants. The results are expressed as average ± standard deviation from 2 technical replicates (n=2 PBMC donors), and one-way ANOVA followed by Dunnett's correction was used to calculate *P*-value, *$P < 0.05$; **$P < 0.005$.

Supplementary Figure S7. Differentially expressed genes (DEG) between SK-MEL-5 educated M-MDSCs and monocytes. (**A-F**) Gene expression levels (normalized values, Log2) of CXCL10, CCL2-MCP1, CCL22-MDC, CD86, STAT6, and CD163 in M-MDSCs and monocytes (n=4 PBMC donors). All with $P < 0.0001$ except P-value 0.0062 for CD86 (t-test).