**Supplementary figure 1. Detection of MDSC subsets in whole blood and in PBMC.** PBMCs were separated on Histopaque with density: 1.077 g/ml (Sigma) at 240 g, 30 min, and room temperature. The whole blood specimen and PBMC were stained with CD45-PB, CD11b-APC, CD33-FITC, HLA-DR-PC5, CD14-PE, CD15-KO and CD16-PC7 (Beckman Coulter) antibodies, red cells were lysed and the samples were analyzed on a Beckman Coulter Navios flow cytometer. **Case 1:** MDSC detection in peripheral blood of a carcinoma patient before (whole blood) and after PBMC separation. a, b Scattergram from hematology analyser Sysmex XN-1000 shows imperfect depletion of neutrophils and eradication of eosinophils in b PBMC sample compared to a whole blood sample. c, d Detection of PMN-MDSC from PBMC as CD15⁺ CD11b⁺ cells according to [9]. e Detection of M-MDSC from PBMC as CD14⁺ HLA-DRlo/cells according to [9]. f Projection of PBMC-derived M-MDSC (blue) and PMN-MDSC (purple) on SSC vs CD11b scattergram. g, h Detection of M-MDSC and CD33hi PMN-MDSC in CD45⁺ CD11b⁺ CD33hi HLA-DRlo/hi (gating not shown) in g whole blood and h PBMC. **Case 2:** MDSC detection in peripheral blood of cancer patient treated with rhG-CSF (filgrastim) before (whole blood) and after PBMC separation. i, j Scattergram from hematology analyser Sysmex XN-1000 shows imperfect depletion of neutrophils and separation of immature granulocytes into in j PBMC sample compared to i whole blood sample. k, l Detection of PMN-MDSC from PBMC as CD15⁺ CD11b⁺ cells according to [9] showing that all immature granulocyte are detected as PMN-MDSCs. m Detection of M-MDSC from PBMC as CD14⁺ HLA-DRlo/cells according to [9]. n Projection of PBMC-derived M-MDSC (blue) and PMN-MDSC (purple) on SSC vs CD11b scattergram. o, p Detection of M-MDSC and CD33hi PMN-MDSC in CD45⁺ CD11b⁺ CD33hi HLA-DRlo/hi (gating not shown) in o whole blood and p PBMC. Quantification of absolute count of M-MDSC and CD33hi PMN-MDSC subset in 5 cases has shown very good recovery of M-MDSC after PBMC separation but variable loss of CD33hi PMN-MDSC during gradient centrifugation (data not shown). APC – Allophycocyanin, FITC – Fluorescein isothiocyanate, KO – Krome orange, PB – Pacific blue, PC5 – Phycoerythrin-Cyanine 5.1, PC 7 – Phycoerythrin-Cyanine 7, PE – Phycoerythrin.
Supplementary figure 2. Gating strategy for multicolor flow cytometry of M-MDSC and CD33\textsuperscript{hi} PMN-MDSC subsets in fresh whole blood. The whole blood specimen was stained with CD45-PB, CD11b-APC, CD33-FITC, HLA-DR-PC5, CD-14-PE, CD15-KO and CD16-PC7 (Beckman Coulter) antibodies, red cells were lysed and the samples analyzed on a Beckman Coulter Navios flow cytometer. MDSCs were determined as CD45\textsuperscript{+} HLA-DR\textsuperscript{-}CD33\textsuperscript{hi} and CD11b\textsuperscript{+} markers. This strategy enables detection of M-MDSC and CD33\textsuperscript{hi} PMN-MDSC subsets as CD14\textsuperscript{+} CD15\textsuperscript{-} monocytic MDSCs (M-MDSC) and CD14\textsuperscript{-} CD15\textsuperscript{+} polymorphonuclear MDSCs (CD33\textsuperscript{hi} PMN-MDSC) [9, 16, 17]. White blood cells were detected as CD45\textsuperscript{+} cells and relative MDSC counts were calculated as the percentages of defined cell populations within CD45\textsuperscript{+} cells. Absolute MDSC count was calculated from WBC count determined by hematology analyzer Sysmex XN-1000. a Healthy control. b Carcinoma patient. For MDSC morphology assessment, MDSC subtypes were purified using fluorescence-activated cell sorting (FACS) with the gating strategy described above. After cell sorting, cell preparation (cytospin) on microscope slide was stained using panoptic staining (Wright-Giemsa). M-MDSCs cells have a morphology similar to monocytes, while CD33\textsuperscript{hi} PMN-MDSCs cells have segmented nuclei similar to segmented granulocytes. In SSC vs CD45 scattergram, CD33\textsuperscript{-}CD11b\textsuperscript{-}CD14\textsuperscript{-}CD15\textsuperscript{-} M-MDSCs (blue) are found in the population of monocytes, while CD33\textsuperscript{hi} CD11b\textsuperscript{+} CD14\textsuperscript{-} CD15\textsuperscript{+} PMN-MDSCs (purple) are located in the granulocyte population. In SSC vs CD16 scattergram, M-MDSCs are negative for CD16, CD33\textsuperscript{hi} have low positivity for CD16. Reproducibility was assessed for absolute M-MDSC and CD33\textsuperscript{hi} PMN-MDSC counts, providing coefficient of variation of 6-11\% for Mo-MDSCs and 25-44\% for CD33\textsuperscript{hi} PMN-MDSCs (data not shown). APC – Allophycocyanin, FITC – Fluorescein isothiocyanate, KO – Krome orange, PB – Pacific blue, PC5 – Phycoerythrin-Cyanine 5.1, PC 7 – Phycoerythrin-Cyanine 7, PE – Phycoerythrin.