Supplementary Material for
CLINICAL RELEVANCE AND SUPPRESSIVE CAPACITY OF HUMAN MDSC SUBSETS

Supplementary Methods:

Upregulation of maturation markers on immature PMN-MDSC
PMN-MDSC subsets were stained and sorted according to the expression of CD11b and CD16. Sorted $CD11b^-/CD16^-$ cells were cultured in 96 well round bottom plates in RPMI 1640 medium (Thermo Fisher scientific, Karlsruhe, Germany) supplemented with 10% (v/v) heat-inactivated FCS, 100 IU/ml penicillin, and 100 mg/ml streptomycin (Thermo Fisher Scientific) in the presence of 40ng/mL G-CSF (Granocyte 34 , CHUGAI PHARMA, Frankfurt/Main , Germany). Medium was exchanged every 2-3 days and cells were stained on day 7 for expression of CD11b and CD16.

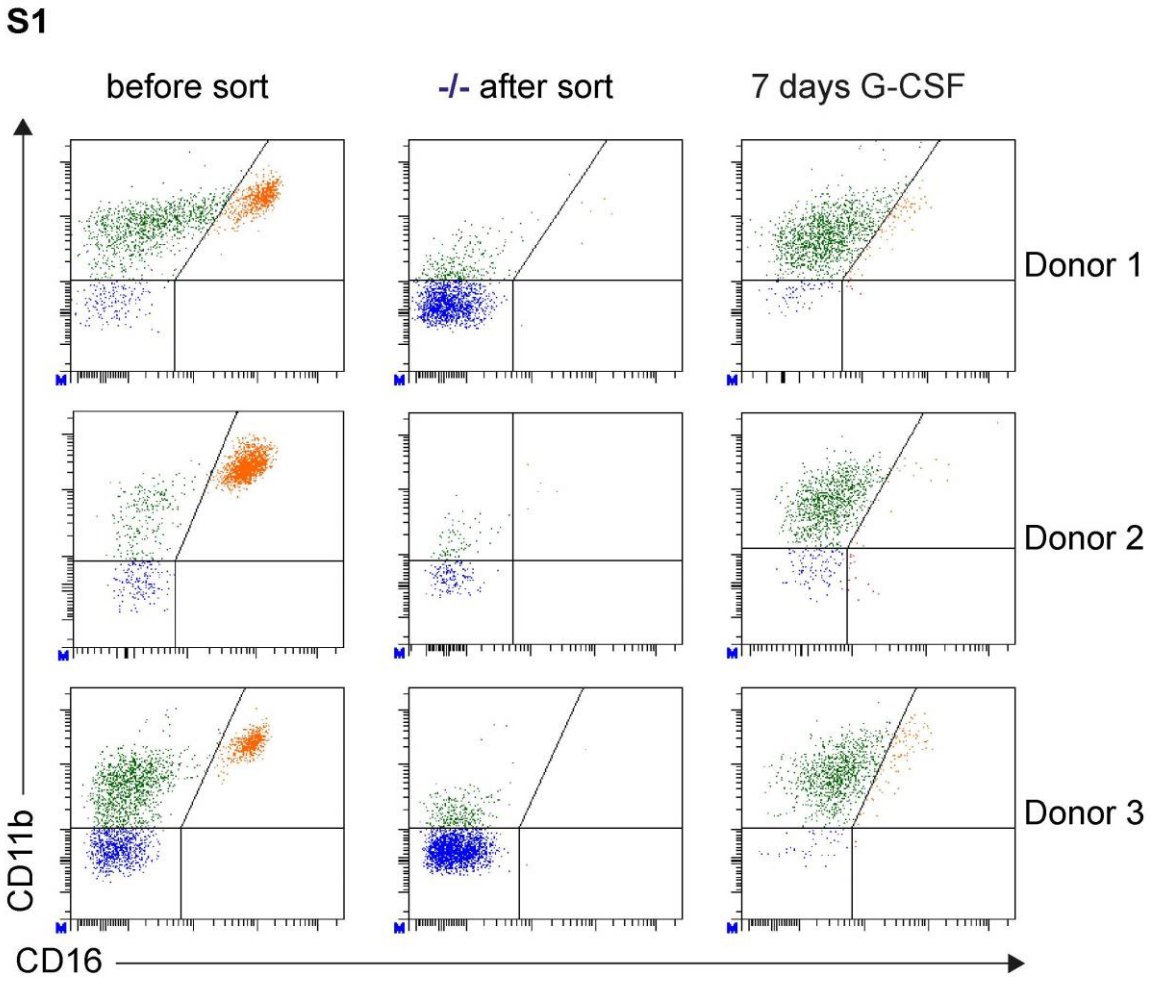

Figure S1: In vitro differentiation of immature PMN-MDSC

CD11b-/CD16- immature PMN-MDSC of three cancer patients (donor 1-3) were sorted as described in figure 4. Cells were cultured for 7 days in the presence of 40ng/mL G-CSF and stained for maturation markers CD11b and CD16. Flow cytometry dot plots of PMN-MDSC before sorting (left column), after sorting (middle column) and after 7 days of differentiation (right column) are shown. Note the increase in CD11b and CD16 expression during in vitro differentiation.

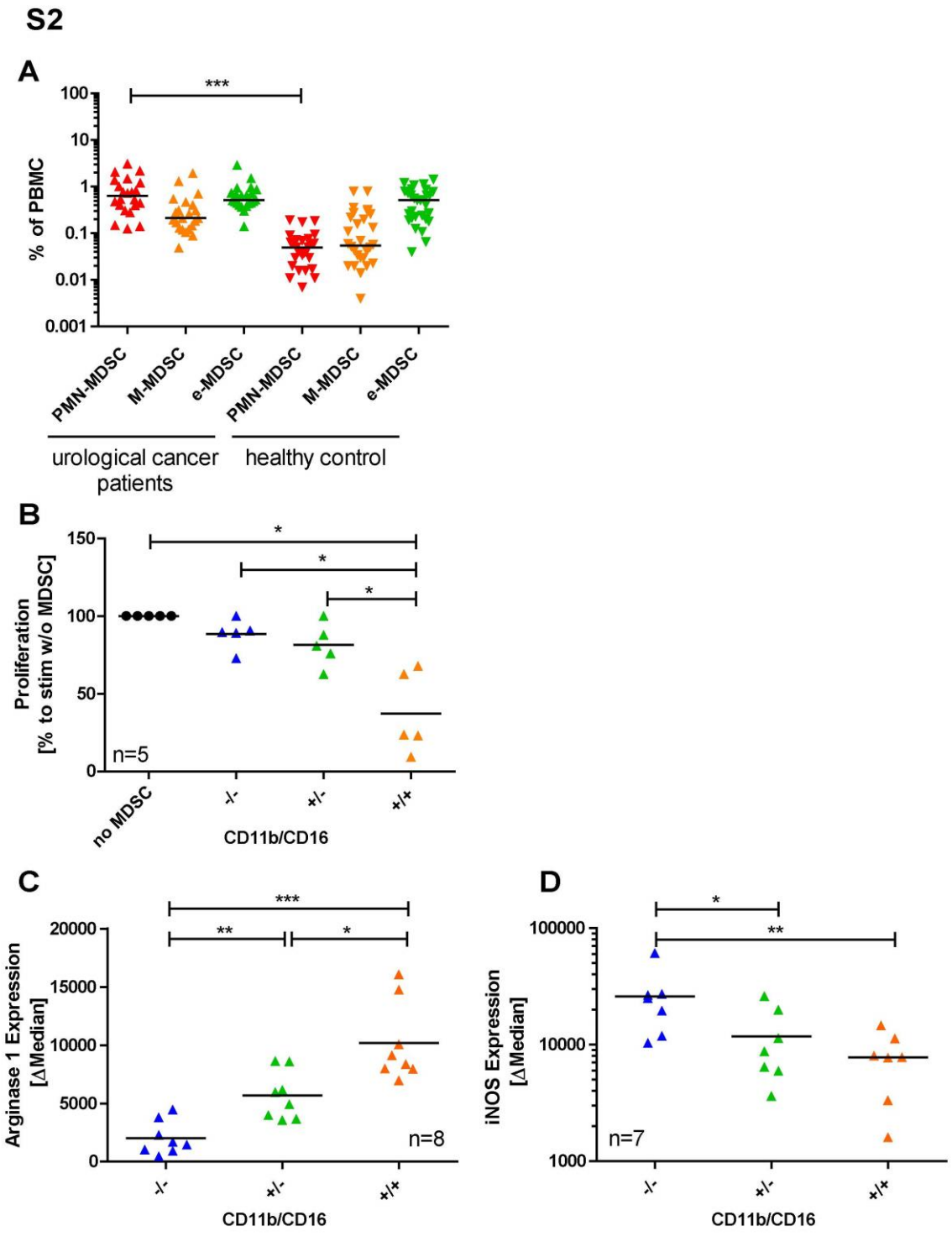

Figure S2: MDSC in patients with urological cancers

(A) MDSC subsets were identified and classified as described in Figure 1A in PBMC of 22 patients with urological cancers and 30 healthy donor controls. Kruskal-Wallis and Dunn´s multiple comparison test were used for statistical analysis. Results were considered significant at *p≤0.05, ** p≤0.005 and *** p≤0.0005. (B) Mature and immature PMN-MDSC subsets were sorted from PBMC of urological cancer patients as described in Figure 4. Suppression assay was performed with autologous CD3+ responder T cells stimulated with plate-coated CD3 and CD28 mAb in the presence of PMN-MDSC subsets (T cell

: MDSC ratio of 2.5:1). Lymphocyte proliferation was measured at day 4. Relative proliferation to stimulated T cell without PMN-MDSC of individual donors is shown. Expression of arginase 1 (C) and iNOS (D) was determined by intracellular FACS staining. Δ Median expression (difference to isotype control) is shown. Wilcoxon signed rank test was used for statistical analysis. Results were considered significant at *$p \leq 0.05$, ** $p \leq 0.005$ and *** $p \leq 0.0005$