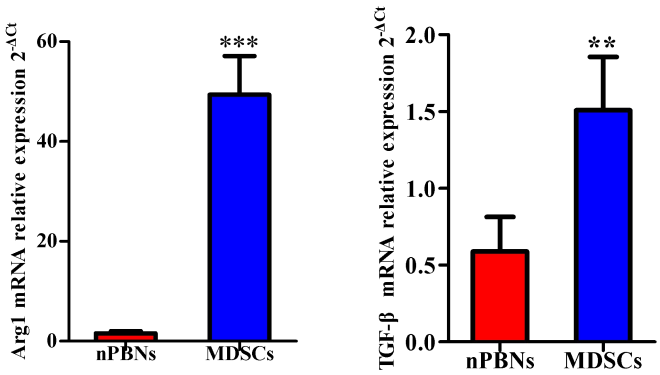

Figure S1
The relative expression of Arginase(Arg)1 and TGF-β mRNA in nPBNs and MDSCs.

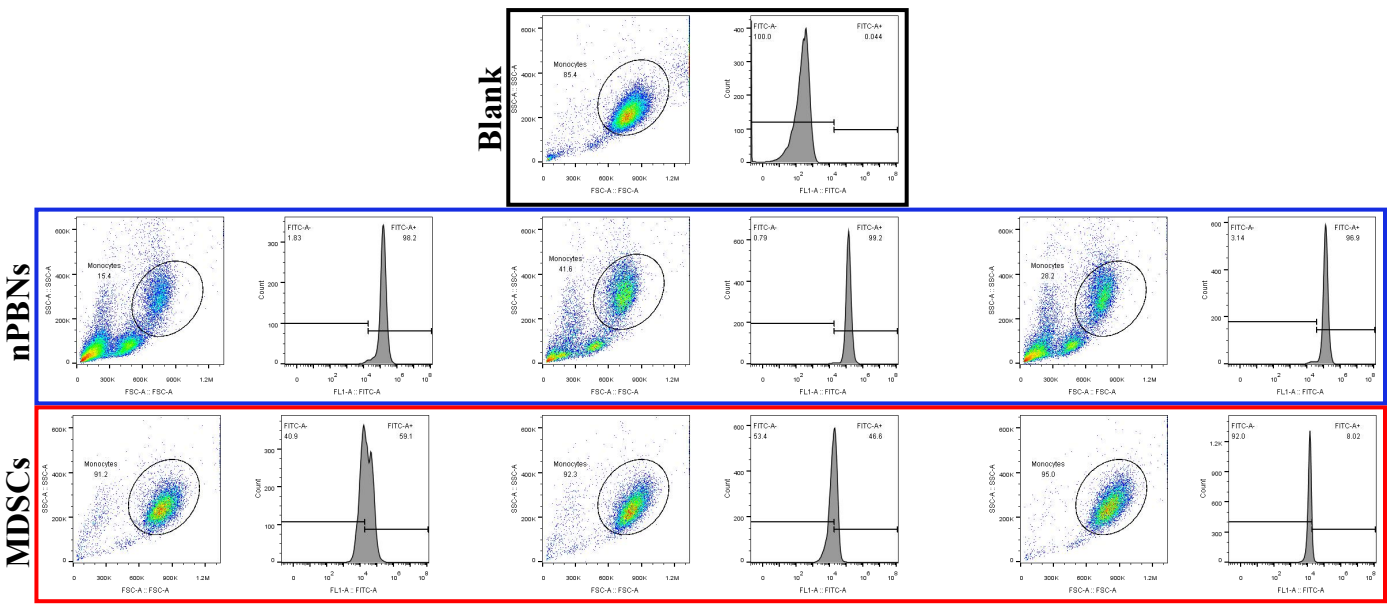

Figure S2 ROS in MDSCs and nPBNs. FITC-DCFH. nPBNs and MDSCs were isolated from balb/c mice and incubated with 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Beyotime).

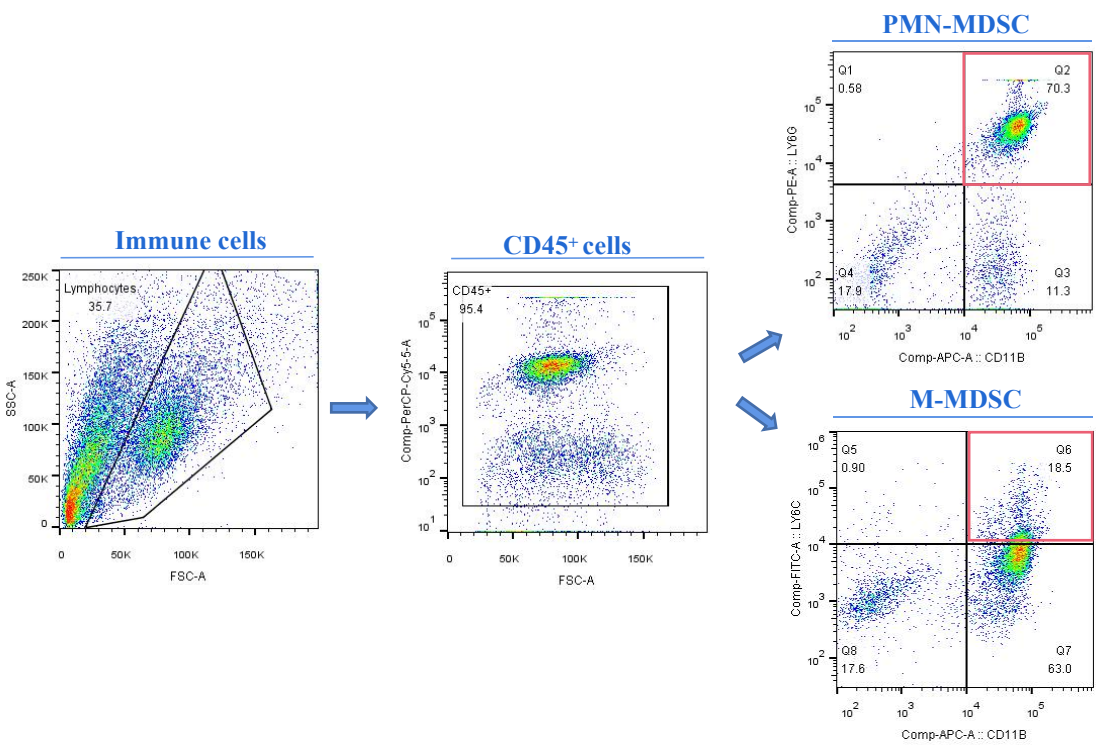

Figure S3 Gating strategy for the MDSC subsets.
PMN-MDSC: $CD11b^{+}Ly6G^{+}$ M-MDSC:$CD11b^{+}Ly6C^{+}$

Library preparation, and Illumina Hiseq xten/Nova seq 6000 Sequencing

RNA-seq transcriptome librariy was established following TruSeqTM RNA sample preparation Kit from Illumina (San Diego, CA) with 1μg of total RNA. First, messenger RNA(mRNA) was isolated according to polyA selection method by oligo(dT) beads and fragmented by fragmentation buffer. Secondly, a SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA) was used to synthesize double-stranded cDNA with random hexamer primers (Illumina).

Next, the synthesized cDNA was subjected to end-repair, and phosphorylation and 'A' base addition following Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantified by TBS380, paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq xten/NovaSeq 6000 sequencer (2 × 150bp read length).

Read

SeqPrep(https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) wereused to trim and quality control for the raw data. With HISAT2 (http://ccb.jhu.edu/software/hisat2/index.shtml)[1]software, clean reads were separately aligned to reference genome withorientation mode.

Differential expression analysis and functional enrichment

The transcript expression level was calculated according to the TPM (transcripts per million) reads method. Quantify gene abundances were analyzed by RSE

M(http://deweylab.biostat.wisc.edu/rsem/)[2]. Notably, differential expression analysis was completed using the DESeq2[3] (p value ≤ 0.05, DEGs(differential expression genes) with |log2FC|>1 and p value ≤0.05 /p value ≤ 0.001 were considered to be significantly). KEGG pathway analysis were carried out by KOBAS(http://kobas.cbi.pku.edu.cn/home.do)[4].

References

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In vivo assays for tumor growth

For bearing mice, 3×10^6 BNL was alone or co-injected with 3×10^5MDSCs isolated from the peripheral blood of BNL-bearing balb/c mice into the subcutaneous space of the right flank of corresponding mice(MDSCs : BNL cells = 1:10 ratio). After 1 week of tumor engraft, mice bearing tumor were equally divided into groups by their tumor volume. MDSCs were injected into the tumor at the same amount weekly for 3 weeks from day 7 after injection. Control mice received an equal volume of saline injections. Mice were treated with vehicle solution(10% dimethyl sulfoxide+40% polyethylene glycol 300+5%Tween-80+45%saline, 10mL/kg) or sorafenib(30 mg/kg) by gavage when the xenografts were palpable from day 7 after inoculation. For MDSCs depletion-treatment experiments, The mice were treated with intraperitoneal injection of 100 μg anti-Ly6G twice a week.

Cell isolation of tumor tissue

Tumor tissues were sliced into 1 mm^3 pieces and incubated with RPMI 1640 medium containing collagenase IV (0.1mg/ml, Worthington) and DNase I (1 mg/ml; Roche) at 37 °C for 30 min. Dissociated cells of tumor tissue were filtered through a 70-μm cell strainer (BD Falcon). Red blood cells were then lysed in ACK lysis buffer at room temperature for 2 min, washed 2 times, and resuspended in precool PBS at 1×10^6 cells/mL for Flow cytometric.

Flow Cytometry

Fc-receptor blocking reagent (Miltenyi Biotec) was used to block the Fc-receptors on tumor cells, and a Percp/Cy5.5-CD45.2 antibody (Biolegend) marked nucleated

hematopoietic cells. Allophycocyanin (APC)-CD11b, fluorescein isothiocyanate (FITC)-Gr-1, phycoerythrin (PE)-ly6G, FITC-Ly6C antibody (Biolegend) were used to identify MDSC subgroups.

ROS measurement

ROS Kit(Beyotime, China)were appropriated to detect apoptotic cells and the expression of ROS as described previously. In particular, nPBNs and MDSCs were isolated from balb/c mice and incubated with 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Beyotime) for 30 min. Stained cells were analyzed using the FACS Analyzer (BECKMAN). The results were analyzed with FlowJo10.0 software (FlowJo).