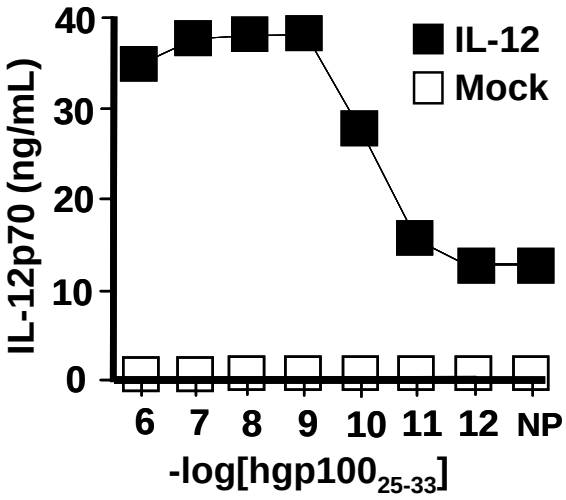
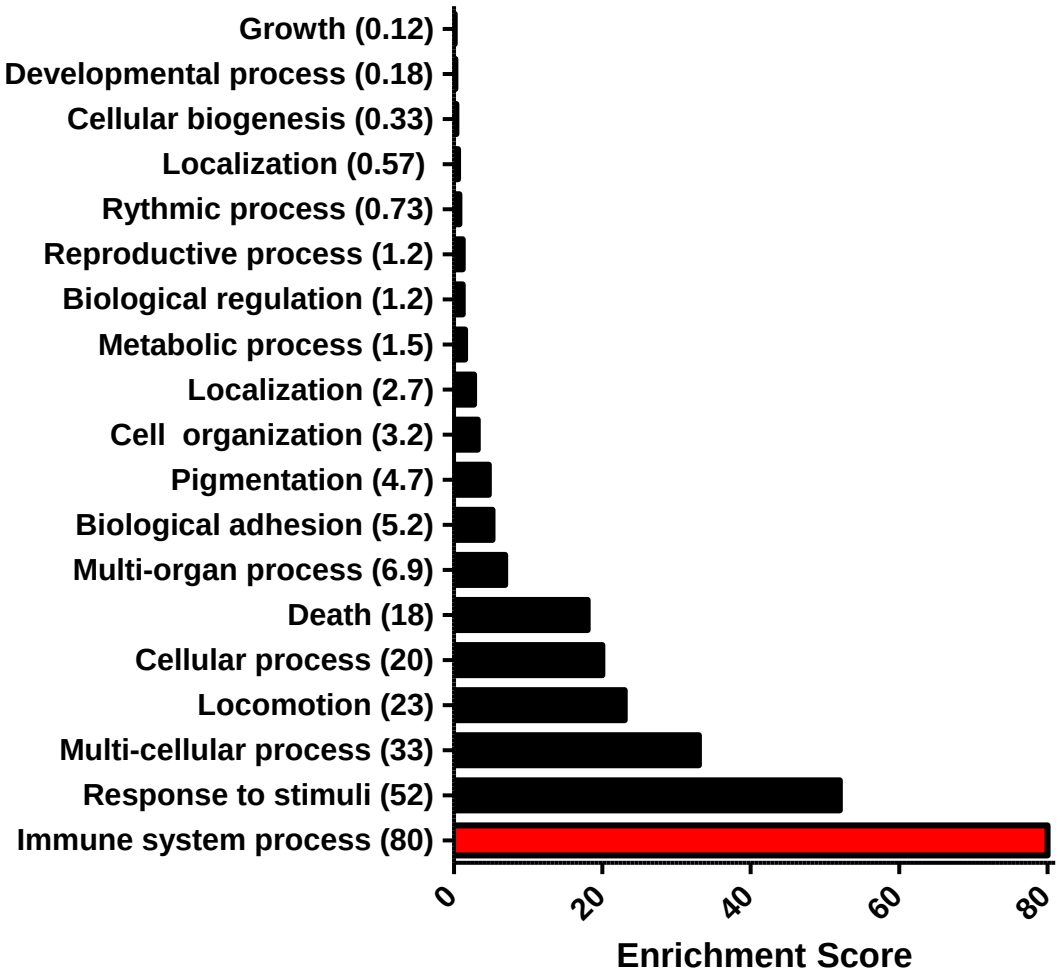
Supplementary Figure 1



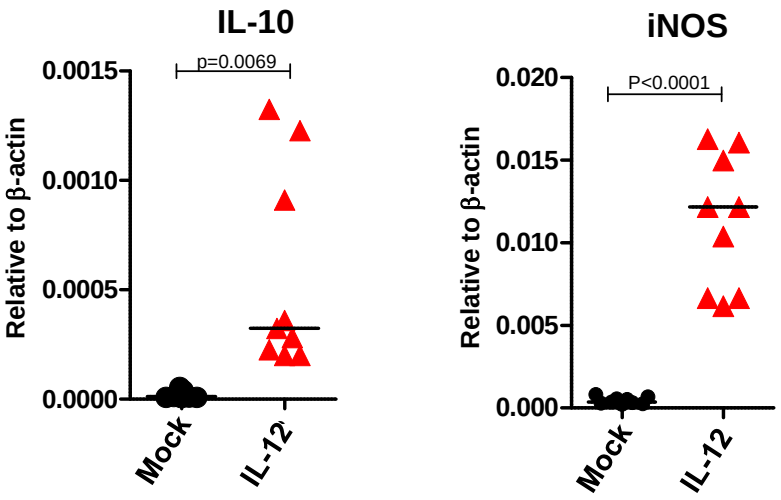
Production of IL-12p70 by pmel-1 $CD8^+$ T cells transduced with a gamma-retrovirus encoding the sequence for a single-chain IL-12 driven by a long-terminal repeat promoter (IL-12 cells). Pmel-1 splenocytes were stimulated with 1 µM hgp100$_{25–33}$ and underwent either mock or single-chain IL-12 transduction 24 hours after stimulation. Four days following transduction, cells were stimulated for 12-18 hours with C57BL/6 splenocytes pulsed with control nucleoprotein peptide or with hgp100$_{25–33}$ peptide at various concentrations. Supernatants from cell cultures were assessed by enzyme-linked immunosorbent assay for IL-12p70 production. Data is representative of 2 independent experiments.

Supplementary Figure 2



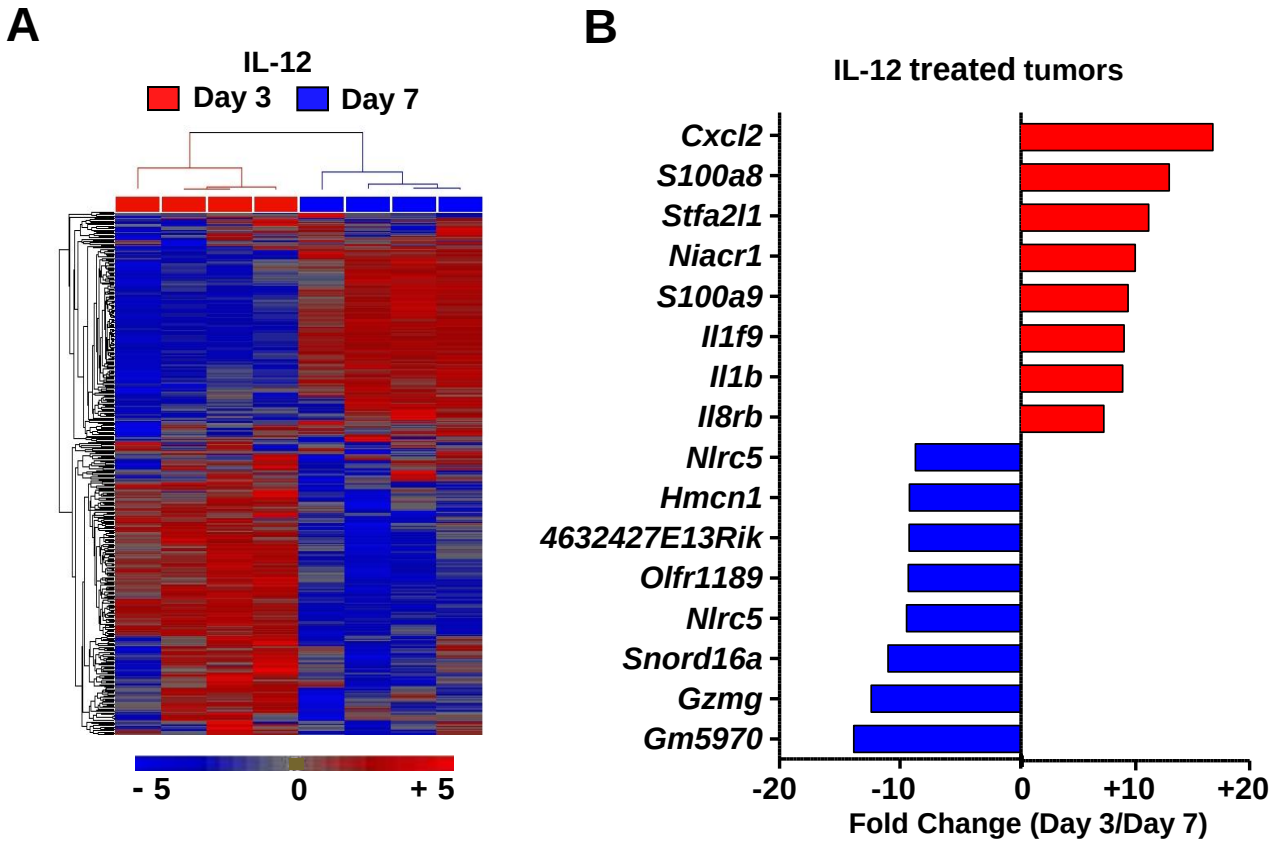
Unbiased gene-ontology enrichment analysis for pathways from B16 tumors of sublethally irradiated mice treated with 10^5 IL-12 cells compared with Mock cells (n=4). Analysis done on differentially expressed genes on both day 3 and 7 after treatment and were identified by One-Way Repeated Measures ANOVA ($p<0.01$) corrected by Benjamini-Hochberg's False Discovery Rate method ($p<0.05$). This gene list was further filtered for between-group alpha levels of $p<0.01$ and a fold change criterion of >2.0 for genes differentially expressed from tumors treated with IL-12 cells over Mock cells.

Supplementary Figure 3



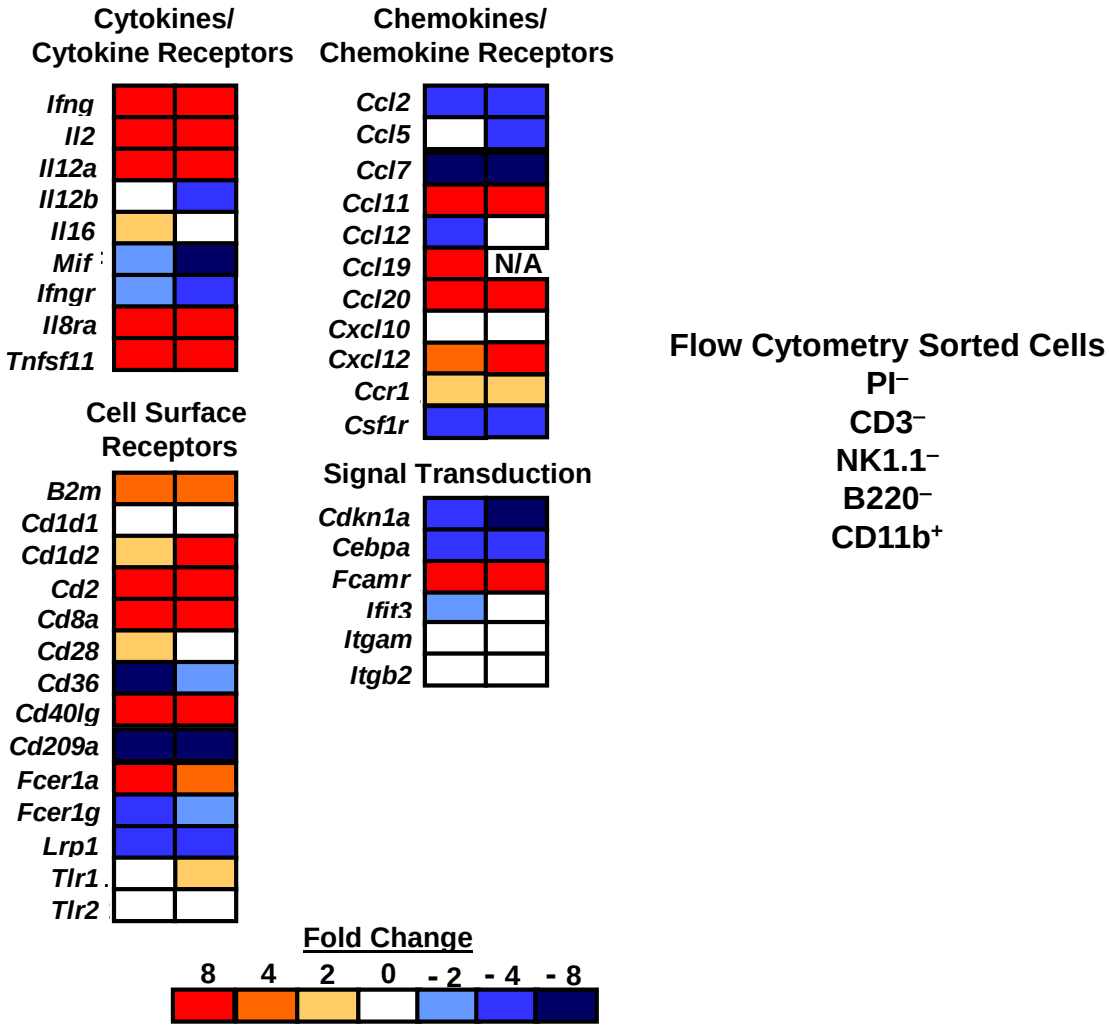
Gene array validation: taqman based PCR for IL-10 and iNOS from B16 tumors of mice 7 days following treatment with 10^5 IL-12 cells compared with Mock cells (n=9). Two tailed student t tests used for statistical analysis.

Supplementary Figure 4



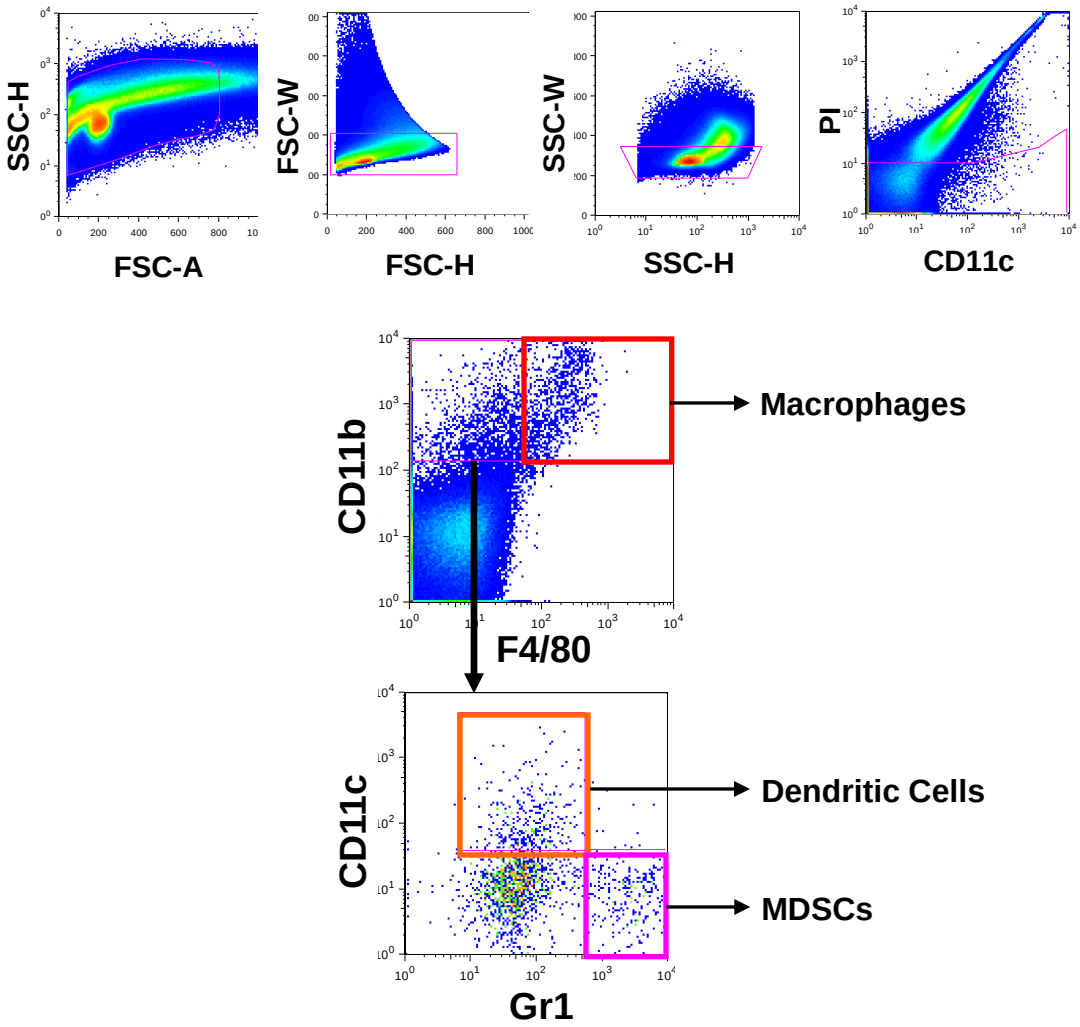
Tumors from mice treated with IL-12 cells display a dynamic gene-expression profile. (**A**) Dendrogram of whole transcriptome analysis displaying the 2776 gene transcripts uniquely and differentially expressed in B16 tumors between day 3 and day 7 following the adoptive transfer of 10^5 IL-12 cells into sublethally irradiated mice. (**B**) The eight most differentially up-regulated and down-regulated genes on day 3 compared to day 7 for B16 tumors from mice treated with 10^5 IL-12 cells. Analysis done on uniquely differentially expressed genes between day 3 and 7 after IL-12 treatment and were identified by One-Way Repeated Measures ANOVA ($p<0.01$) corrected by Benjamini-Hochberg's False Discovery Rate method ($p<0.05$). This gene list was further filtered for between-group alpha levels of $p<0.01$ and a fold change criterion of > 2.0 for genes differentially expressed from tumors treated with IL-12 on day 3 compared to day 7. All data are representative of 4 independent samples.

Supplementary Figure 5



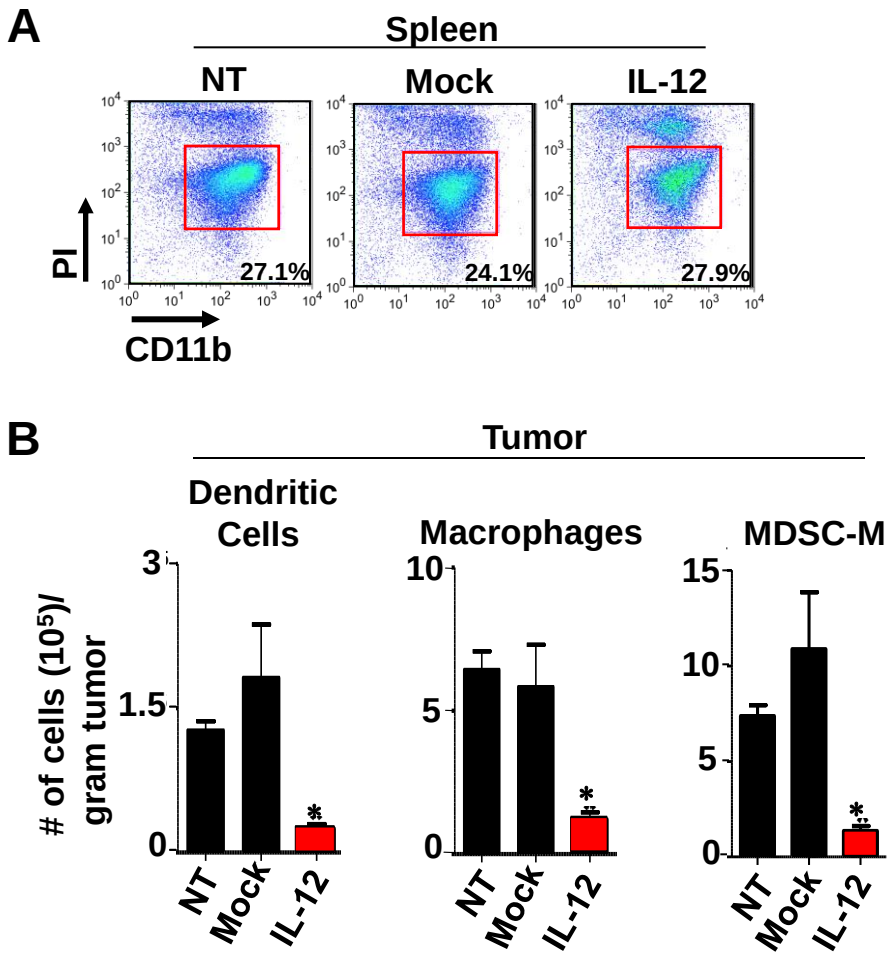
CD11b$^+$ myeloid cells from tumors of mice treated with IL-12 compared with Mock cells display a distinct gene-expression profile. PCR based array for transcripts common to antigen-presenting cells from flow cytometry sorted PI$^-$, CD3$^-$, NK1.1$^-$, B220$^-$, CD11b$^+$ cells from B16 tumors of sublethally irradiated mice 7 days following treatment with IL-12 cells compared to Mock cells. Each column represents an independent experiment (n=2) of cells sorted from tumors pooled from 4-5 separate mice. Data is expressed as a fold change in gene expression for select transcripts from CD11b$^+$ cells from tumors of mice treated with IL-12 cells divided by gene expression for select transcripts from CD11b$^+$ cells from tumors of mice treated with Mock cells.

Supplementary Figure 6



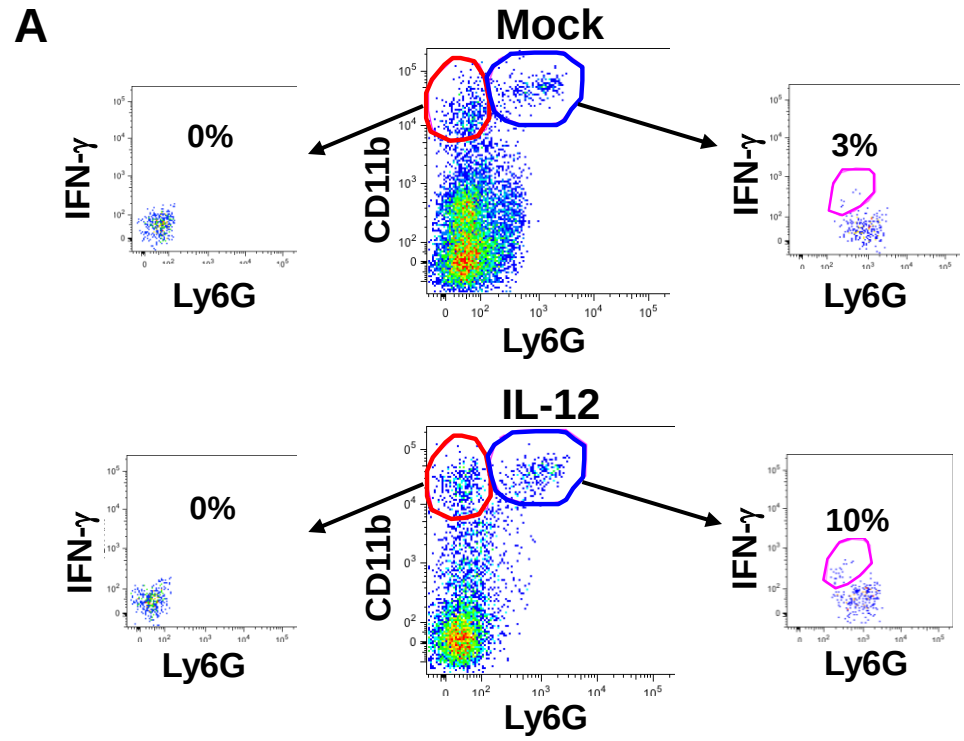
Gating strategy for isolation of macrophages, dendritic Cells and MDSCs from B16 tumors. Five days following the treatment with IL-12 or Mock cells, B16 tumors were harvested into a single cell suspension, stained for PI, CD11b, CD11c, F4/80, Gr1, and sorted by flow cytometry into collection tubes for $CD11b^{+}$ $F4/80^{Hi}$ macrophages, $CD11b^{+}$ $CD11c^{Hi}$ dendritic cells, and $CD11b^{+}$ $Gr1^{Hi}$ myeloid-derived suppressor cells. Flow cytometry plots are representative gates for all independent sorting experiments.

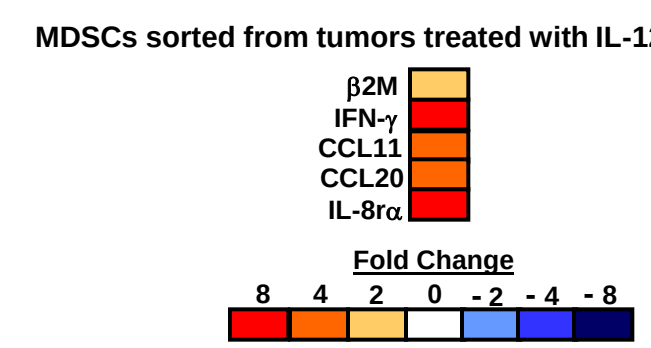
Supplementary Figure 7



C56BL/6 mice bearing B16 tumors treated with IL-12 cells compared with Mock cells display decreased numbers of myeloid-derived cells in tumors but not spleens. (**A**) Representative flow cytometry plot for $CD11b^{+}$ cells from spleens of mice 1 week following treatment with IL-12 cells compared with Mock cells (n=3). (**B**) In mice treated similarily to (**A**), single cell tumor supsensions were created for the enumeration and quantification of $CD11b^{+}$ $CD11c^{Hi}$ dendritic cells, $CD11b^{+}$ $F4/80^{Hi}$ macrophages, and $CD11b^{+}$ $Ly6C^{Hi}$ $Ly6G^{Low}$ myeloid-derived suppressor cells.

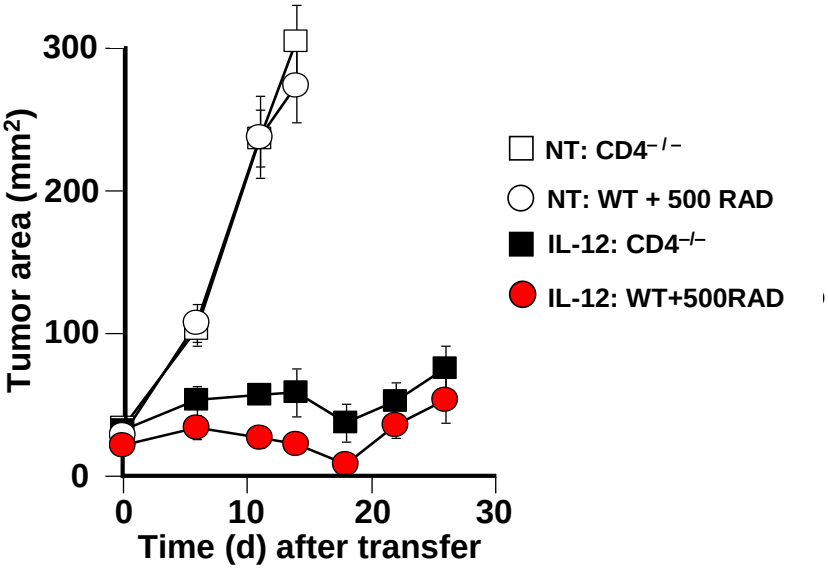
Supplementary Figure 8





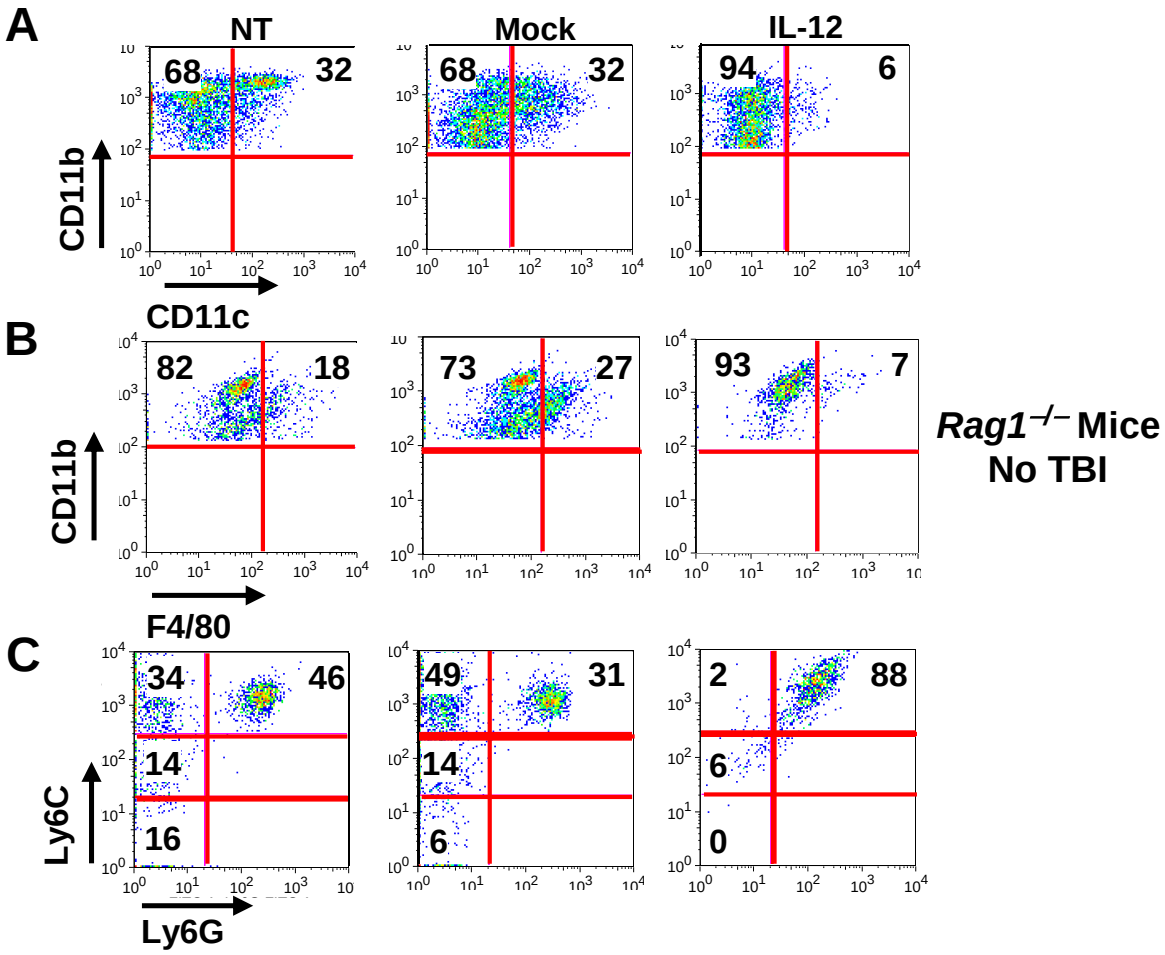
Increased IFN-γ expression in CD11b^{+} Ly6G^{Hi} MDSC/Neutrophils following treatment with IL-12 compared with Mock cells. (**A**) Flow cytometry plots of single cell tumor suspensions harvested 6 days following cell transfer display an increase in the percentage of IFN-γ positive CD11b^{+} Ly6G^{Hi} cells within tumors from IL-12 treated mice. No CD11b^{+}Ly6G^{Low} cells stained positive for IFN-γ. All plots gated on live cells. (**B**) MDSCs from B16 tumors of mice treated with IL-12 or Mock cells were flow cytometry sorted and examined for change in gene expression for select transcripts. Data are expressed as a fold change in gene expression of GR1^{Hi} cells from tumors of mice treated with IL-12 cells divided by the gene expression for select transcripts of GR1^{Hi} cells from tumors of mice treated with Mock cells.

Supplementary Figure 9



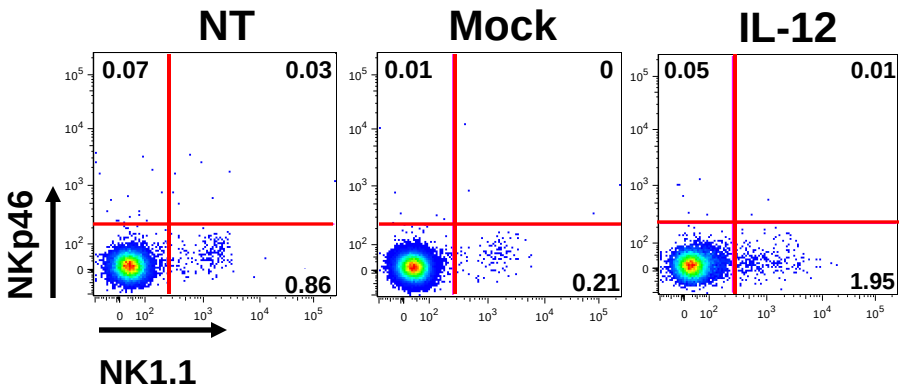
IL-12 cells induce tumor regression in non-ablated mice genetically devoid of $CD4^+$ T cells. Sublethally irradiated C57BL/6 mice (5Gy TBI) or non-irradiated $CD4^{-/-}$ mice bearing 10 day established subcutaneous B16 tumors (n=5/group) were treated with 10^5 IL-12 cells. Data is shown as a mean +/- SEM and is representative of 2 independent experiments.

Supplementary Figure 10



Non-ablated C56BL/6 *Rag1*$^{-/-}$ mice bearing 10 day established B16 tumors were treated with 10^5 IL-12 cells and display a loss of tumor infiltrating CD11b^{+} CD11c^{Hi} dendritic cells, CD11b^{+} F4/80^{Hi} macrophages, and CD11b^{+} Ly6C^{Hi} Ly6G^{Low} but not CD11b^{+} Ly6C$^{Mid\text{-}Hi}$ Ly6G^{Hi} myeloid-derived cells. Single cell tumor suspensions 14 days following treatment of IL-12 or Mock cells were analyzed by flow cytometry for CD11b, F4/80, CD11c, Ly6C, and Ly6G. Panels A and B are gated on live cells, and panel C is gated on live CD11b^{+} cells. All flow cytometry plots are representative of 3 independent samples.

Supplementary Figure 11



B16 tumors from sublethally irradiated mice treated with IL-12 cells do not display an increase in infiltrating innate lymphoid cells. C56BL/6 mice bearing 10 day established B16 tumors were sublethally irradiated and treated with either IL-12 or Mock cells. Flow cytometry plots of single cell tumor suspensions display an increase in the infiltration of NK cells (NK1.1^{+}) but no increase in NKp46^{+} NK1.1^{-} lymphoid tissue–inducer (LTi) cells within tumors of mice treated with IL-12 cells compared to Mock cells. All flow cytometry plots are gated on live cells and are representative of 3 independent experiments.

Adoptive Cell Transfer

Six to twelve week old mice (n=5 for all groups) were injected subcutaneously with 5 x 10^5 B16 melanoma cells or 2 x 10^6 Cloudman S91 melanoma cells. Ten to fourteen days (21-28 days for Cloudman tumors) later, mice were irradiated with 5 Gy total body irradiation (TBI) and given 1 x 10^5 IL-12 transduced pmel-1 $CD8^+$ T cells (IL-12 cells) or 2.5 x 10^5 pmel-1 TCR and IL-12 double transduced C57BL/6 WT or *Ifnγ*$^{-/-}$ $CD8^+$ T cells by tail vein. For NK1.1 depletion experiments, mice were given intraperitoneal injections of 200μg of NK1.1 depleting monoclonal antibody (Harlan Bioproducts) every 3 days starting on the day mice were irradiated (5 Gy) and given IL-12 cells for 3 weeks post treatment. Tumors were measured using digital calipers and the tumor area was calculated as the product of perpendicular diameter by investigators in a blinded manner. All experiments were performed independently at least twice with similar results and all tumor curve data is shown as mean +/– standard error of the mean.

Bone marrow chimeras

C57BL/6 thy1.1$^+$ or C57BL/6 *Il12rb2*$^{-/-}$ mice were lethally irradiated with 6Gy + 6Gy TBI (fractionated over 6 hours) and reconstituted with 5 x 10^6 bulk bone marrow cells isolated from femurs of C57BL/6 thy1.1$^+$ or C57BL/6 *Il12rb2*$^{-/-}$ mice 24 hours after irradiation. Six weeks after bone marrow transplantation, peripheral blood was analyzed for the percentage of thy1.1$^+$ lymphocytes in C57BL/6 *Il12rb2*$^{-/-}$ mice. Approximately 85%-90% of circulating cells were of donor origin based on flow cytometry staining for thy1.1. Six weeks later subcutaneous B16 tumors were implanted and mice were sublethally irradiated with 5Gy TBI prior to adoptive cell transfer of 1x10^5 Mock or IL-12 cells.

Whole genome expression analysis

Subcutaneous B16 tumors were excised from mice 3 and 7 days following treatment with 1 x 10^5 IL-12 or Mock cells and single cell suspensions were resuspended into RLT buffer (Qiagen) and total RNA was isolated using the RNeasy kit (Qiagen). Three hundred nanograms of total RNA were amplified using Ambion WT expressionkit (Invitrogen) according to manual. Fragmented single stranded sense DNA were terminal labeled and hybridized to Mouse GeneChip 1.0 ST array (Affymetrix) and stained on a Genechip Fluidics Station 450 (Affymetrix), all according to the respective manufacturers' instructions. Arrays were then scanned on a GeneChip Scanner 3000 7G (Affymetrix). After Robust Multichip Average (RMA) normalization, differentially expressed genes were identified by One-Way Repeated Measures ANOVA ($p<0.01$) corrected by Benjamini-Hochberg's False Discovery Rate method ($p<0.05$) and this gene list was further filtered for between-group alpha levels of $p<0.01$ and a fold change criterion of >2.0 for genes differentially expressed from tumors treated with IL-12 over Mock cells. The statistically significant gene expression changes were functionally analyzed using Ingenuity Pathways Analysis (Ingenuity Systems) software. This program analyses RNA expression data in the context of known biological responses and regulatory networks and enabled us to generate Figure 5C. For further analysis, we performed an unbiased gene ontology enrichment analysis for biological processes from genes found to be statistically significant. These findings were displayed in Supplementary Figure 2 and Figure 5.

Immunofluorescent Confocal Microscopy

B16 tumors were excised and flash frozen in liquid nitrogen. Cryostat sections were fixed, prepared and stained with primary biotinylated mouse anti mouse CD90.1 (thy1.1) antibody (OX-7 clone, BD Biosciences) and primary purified rat anti mouse CD31 (R35-95 clone, BD Biosciences). Secondary immunofluorescent labeling was accomplished with a FITC (green) conjugated streptavidin antibody (BD Biosciences) and Alexo Flouro 586 labeled anti rat IgG. All slides were mounted with DAPI containing mounting media (Prolong Gold, antifade reagent with DAPI; Invitrogen Corp.). Confocal images were captured using a Zeiss LSM 510 Confocal system (Carl Zeiss Inc, Thornwood, NY) with an Axiovert 100M inverted microscope. Plan Apochromat 1.3 NA oil-immersion (40X) objectives were used at various zoom settings.