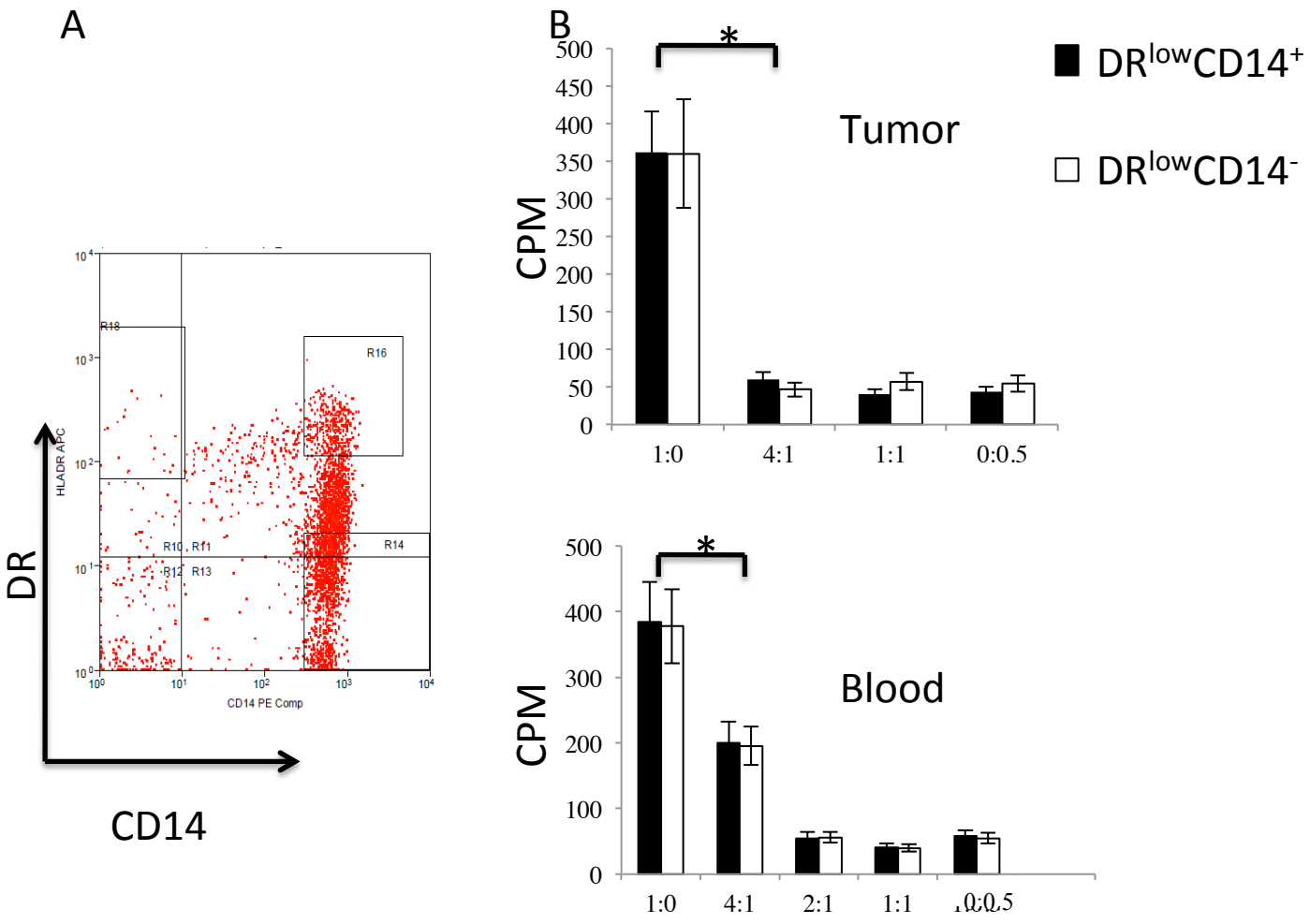
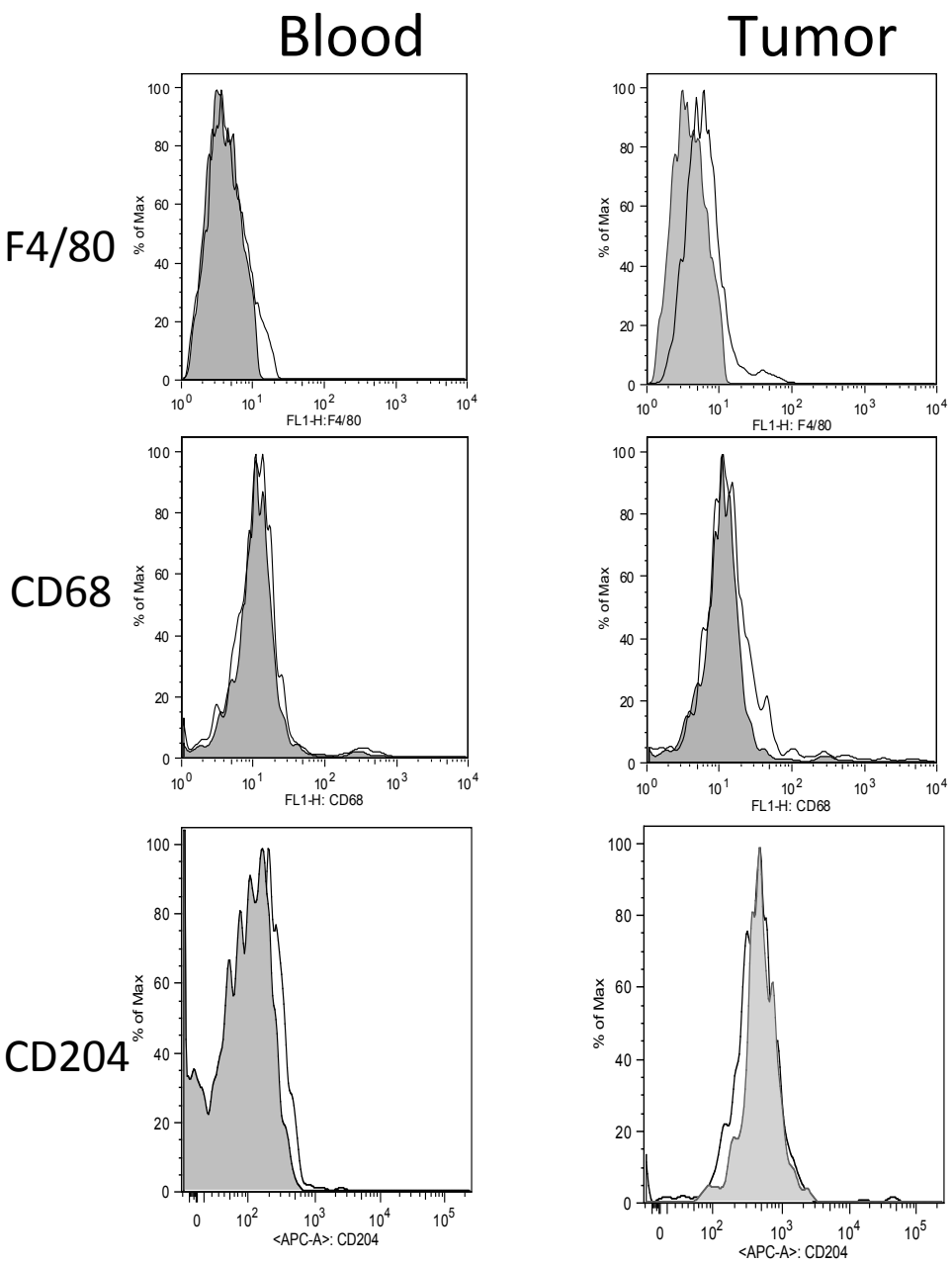
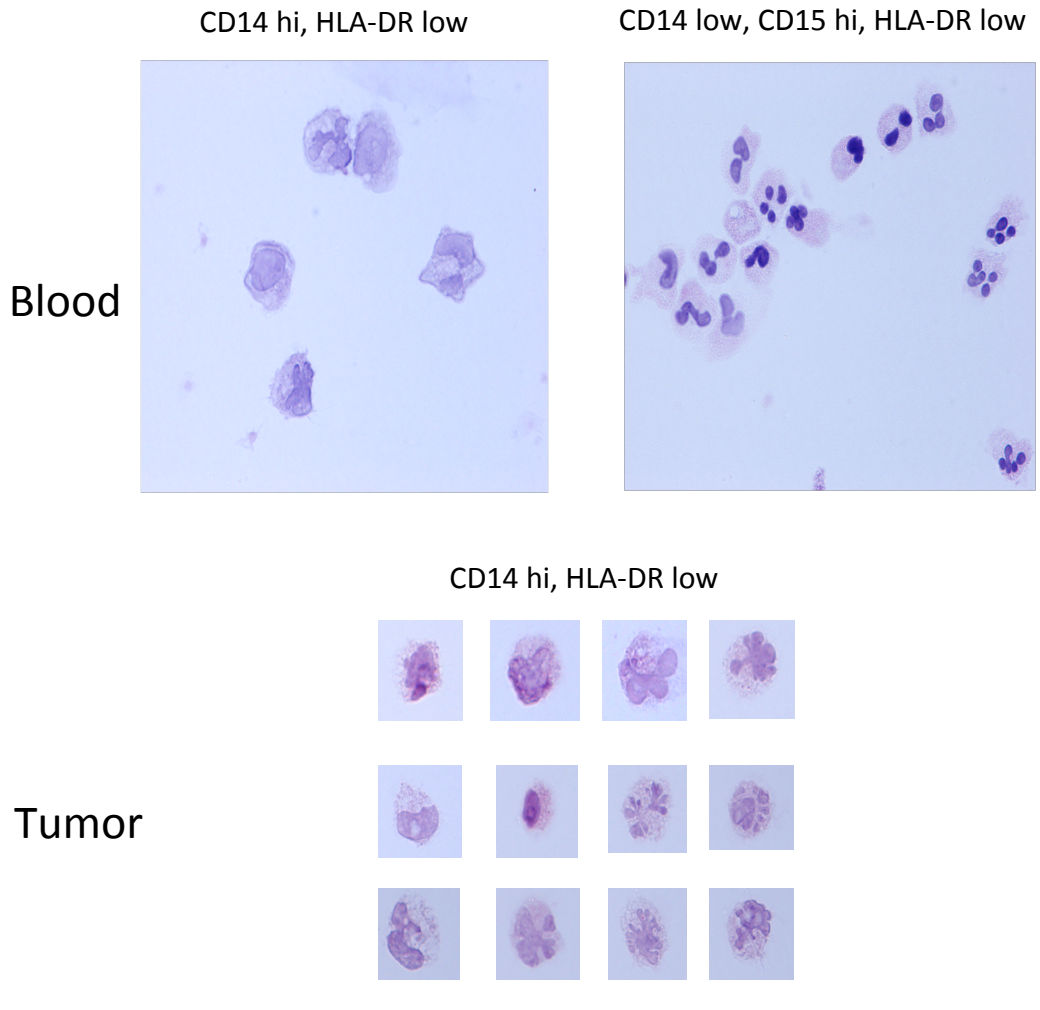


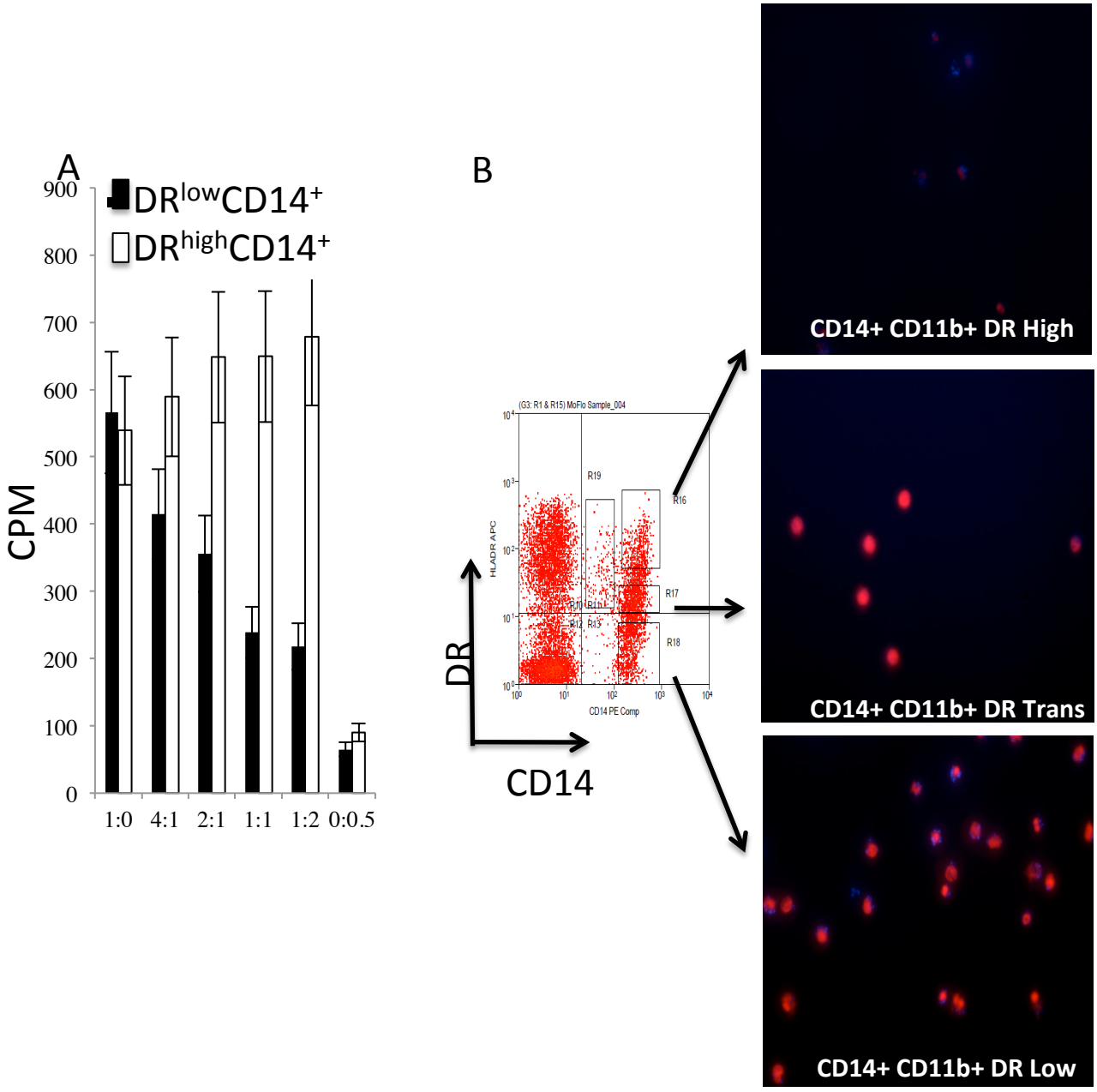
Supplemental Figure 1.



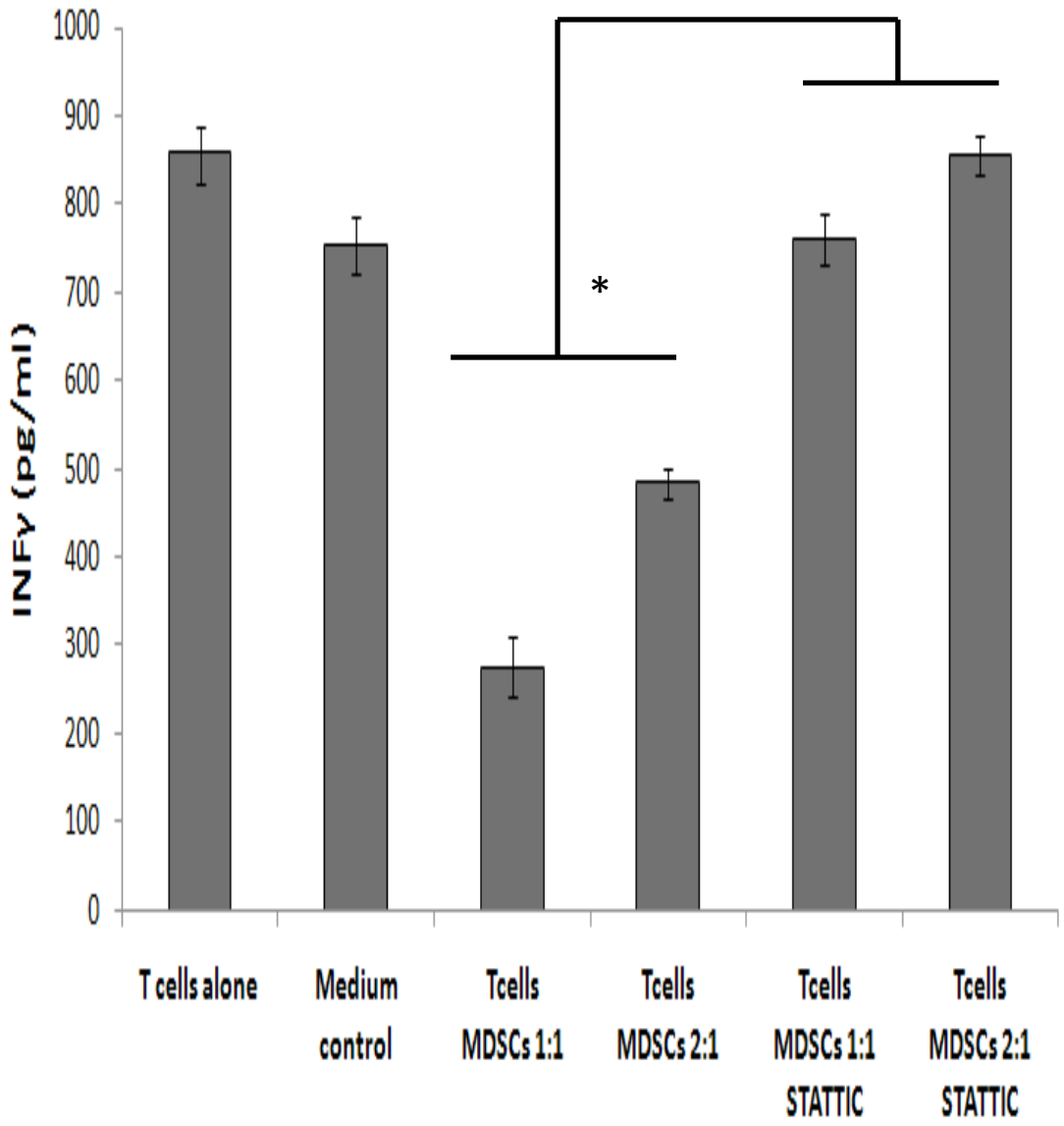
Supplemental Figure 2.



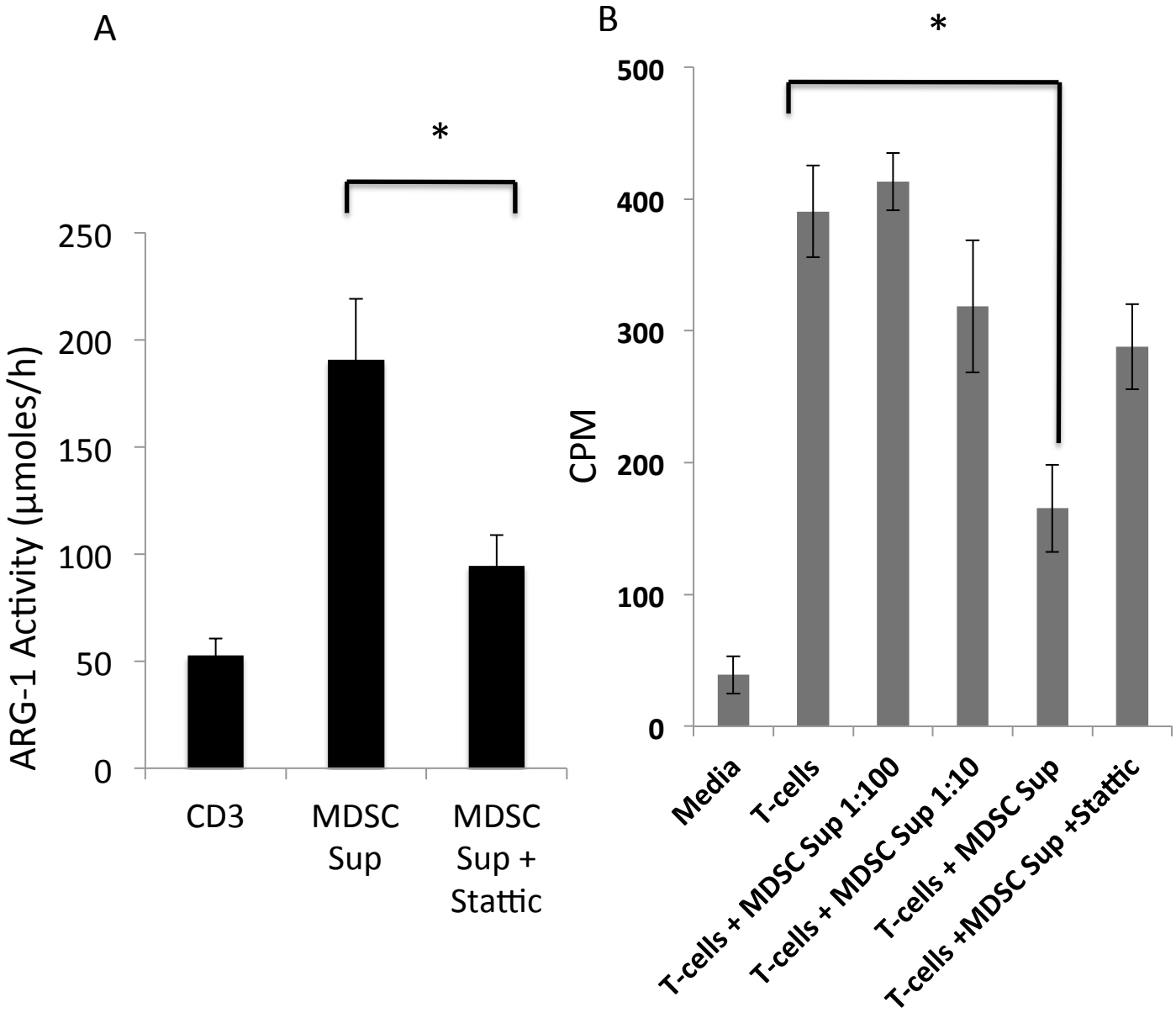
Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Figure 5.



Supplemental Figure 6.

Supplemental Figure 1. ***There are two populations of MDSC cells from HNSCC patients.*** A. Representative FACS of myeloid cells from HNSCC patients obtained during sorting of the tumor specimen. X- axis represents CD14 staining and y-axis represents HLA-DR staining. Both CD14- and CD14+ populations were collected. B. Sorted CD14-DR^{low} and CD14+DR^{low} cells from peripheral blood and tumor were used for suppression assays with autologous T-cells. X-axis label represents ratio of T-cell:MDSC ($p<0.05$,*).

Supplemental Figure 2. ***Human blood and tumor CD14^{+} HLA-DR$^{-/low-}$ MDSC do not express common macrophage markers***. An aliquot of sorted CD14+ HLA-DR$^{-/low}$ MDSC were stained with murine anti-human CD68-Alexa Fluor conjugate (BioLegend), with rat anti-human F4/80-FITC conjugate (Abcam), and with mouse anti-human CD204-APC conjugate (R and D systems). Isotype antibodies were used as controls (shaded histograms).

Supplemental Figure 3. ***Human CD14^{+} HLA-DR$^{-/low-}$ cells are suppressive MDSC in comparison to DR^{high} myeloid cells.*** A. T-cell suppression assays were performed with CD14+ DR^{low} using CD14+DR^{high} as the myeloid control cells. In some cases, CD14+DR^{high} increased the proliferative potential of autologous T-cells as shown. B. Representative FACS from HNSCC patients obtained during sorting of the peripheral blood. Sorted CD14+ cells were fractionated and their ROS level were analyzed using DHE staining (original magnification, x200).

Supplemental Figure 4. ***CD14+ HLA-DR$^{-/low-}$ MDSC are monocytic cells, while CD14- HLA-DR$^{-/low-}$ MDSC have both polymorphonuclear and monocytic cells.*** Sorted cells were fixed onto slides using Cytospin, fixed, and stained with H&E. Tumor CD14+ HLA-DR$^{-/low}$ MDSC cells are mostly monocytic looking similar to CD14^{+} HLA-DR$^{-/low-}$ MDSC from blood, and sorted CD14^{-} HLA-DR$^{-/low-}$ MDSC from peripheral blood displayed more polymorphonuclear

morphology (Original magnification, x600). Tumor MDSC samples were frequently less concentrated and only 1-2 cells could be visualized under a comparable field. To present representative histology from matched specimens, we displayed individual cells from different fields in the tumor compartment. All pictures are from a single patient.

Supplemental Figure 5. ***$CD14^{+}$ $HLA\text{-}DR^{-/low}$ MDSC suppress expression of INFγ from T-cells, and STAT3 inhibition blocks the MDSC dependent decrease in IFNγ expression.*** Autologous T-cells were mixed with MDSC under stimulating conditions identical to ^{3}H-thymidine uptake assays. ELISA was used to quantitate of INFγ in the supernatant.

Supplemental Figure 6. ***Supernatant harvested from cultured HNSCC MDSC has arginase I activity and can suppress T-cells.*** A. Conditioned media from sorted MDSC were harvested over 3 days in vitro and this was used for arginase assay as described in the Methods section. After L-arginine substrate was incubated for 1 hr, the urea concentration was measured at 540 nm. ARG1 assay with control samples with non-conditioned media were subtracted and this activity was decreased in comparison to conditioned media from MDSC treated with STATTIC ($p<0.05$,*). B. Conditioned media from sorted MDSC was incubated with T-cell stimulation assay. Diluted MDSC supernatant decreased the proliferative potential of T-cells (1:10 dilution), but only non-diluted supernatant showed statistically significant T-cell proliferation ($p<0.05$, *). Addition of STATTIC to MDSC decreased the ability of the MDSC supernatant to suppress T-cell proliferation.

Supplemental Table 1. Primer pairs used for ChIP assay. The site #s refers to the potential STAT3 binding sites noted in Figure 6 and Supplemental Table 2.

Supplemental Table 2. Sequence of the human ARG1 promoter region with the 6 potential pSTAT3 binding sites as predicted by the consensus sequences GGAAC. The binding site numbers correspond to the binding site numbers in Figure 6.

Supplemental Table 1. Primer pairs used for ChIP assay. The site numbers refer to the potential STAT3 binding sites noted in Figure 6.

Site #1
5'-GAAGTCAGCATGAGTTCACCAAG-3'
5'-GACATCGTAAGGAAATTTATC-3'

Site #2
5'-GAAATGTGTCTCATGGATTAAC-3'
5'-CGTCCTTGTAGAAGAAGGGCC-3'

Site #3
5'-GATTCTACAATTATTTTCCTG-3'
5'-CATGAGGGTAAATGGTTAATC-3'

Site #4
5'-GTGTCTGATGGACCAGATAAC-3'
5'-CTTGTGTTACATAGTTGCCAC-3'

Site #5
5'-GATGGATTCAGGAACTAAGTG-3'
5'-GAATGCTTTGTGCTTTGGAAG-3'

Site #6
5'-CAAAATGTTTTCCCACCAATAG-3'`
5'-GTCAACCTCTATGCCCCTGAGC-3'

Supplemental Table 2. Sequence of the human ARG1 promoter region (5'to 3') with the 6 potential pSTAT3 binding sites as predicted by the consensus sequences GGAAG.

TAGAAGTCAGCATGAGTTCACCAAGAACTGGACCTGTCAAGGTCAGCCCATC
AACTTTGACAG**GGAAG**^{1}TCAGATTGGCAGAAGAAAAGGTAGTAAAGTG
TGATGAGATTTTATTGAGACACCGGATAAATTTCCTTACGATGTCCTTGTGAA
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CAGAGTGTTTGTAAAAGTGCTGCCTTATTCAACATTTTTATCAGTGACTTGGA
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AAG^{4}CGAGATGTTTTGAAGACAGCGACCAAGTTGGTGACCTGAAGGAAA
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TTATACCCTAAAAGTTTATGAAATGTGTCTCATGGATTAACCATTTACCCTCA
TGTGTGAAATCTCAACTCAGGATTTTAGGGCT**GGAAG**^{2}GGATGTGACA
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GAGGTGTCCTCATTAGATAAAGGTTGTTTATTCAACCCAAGTATAAATGGAA
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AGCAAAGAGAAGTGTCAGAGC**ATG**AGCGCCAAGTCCAGAACCATAGGGAT
TATTGGAGCTCCTT