Supplemental Figure 1.

A

B

CD14

DR

DR\textsuperscript{low}CD14\textsuperscript{+}

DR\textsuperscript{low}CD14\textsuperscript{−}

Tumor

Blood

CPM

1:0 4:1 2:1 1:1 0:0.5

1:0 4:1 2:1 1:1 0:0.5

*
Supplemental Figure 2.
Supplemental Figure 3.

Blood

CD14 hi, HLA-DR low

CD14 low, CD15 hi, HLA-DR low

Tumor

CD14 hi, HLA-DR low
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\text{low} and CD14+DR\text{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\text{+} HLA-DR\text{low} MDSC do not express common macrophage markers.** An aliquot of sorted CD14\text{+} HLA-DR\text{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\text{+} HLA-DR\text{low} cells are suppressive MDSC in comparison to DR\text{high} myeloid cells.** A. T-cell suppression assays were performed with CD14+ DR\text{low} using CD14+DR\text{high} as the myeloid control cells. In some cases, CD14+DR\text{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\text{+} HLA-DR\text{low} MDSC are monocytic cells, while CD14\text{-} HLA-DR\text{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\text{low} MDSC cells are mostly monocytic looking similar to CD14\text{+} HLA-DR\text{low} MDSC from blood, and sorted CD14\text{-} HLA-DR\text{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-DR$^{low}$ MDSC suppress expression of INF$\gamma$ from T-cells, and STAT3 inhibition blocks the MDSC dependent decrease in IFN$\gamma$ expression. Autologous T-cells were mixed with MDSC under stimulating conditions identical to $^3$H-thymidine uptake assays. ELISA was used to quantitate of INF$\gamma$ 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’-CGTCTTGTAGAAGAAGGGCC-3’ |

| Site #3 | 5’-GATTCTACAATTATTTCTCTG-3’  
|         | 5’-CATGAGGGTAAATGGTAAATC-3’ |

| Site #4 | 5’-GTGTCTGTGGACCAAGTAGA-3’  
|         | 5’-CTTGTTACATAGTTGCAC-3’ |

| Site #5 | 5’-GATGGATTCAGGAACCTAAGTG-3’  
|         | 5’-GAATGGCTTTGTGCTTGGGAAG-3’ |

| Site #6 | 5’-CAAAATGTGTTTCCCACCAATAG-3’  
|         | 5’-GTCAACCTCTATGCCCTGAGC-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.

TAGAAGTCAGCATGAGTTCCAACAGAAACTGGACCTGCAAGGTCAGCCCATC
AACTTGACAGGGAAGG
TCAGATTGGCAGAGAAATGGTAGTAAGTGGGATGAGATATTTTGGACAGCACCAGAAGATTAC
CAGGATGAGTTGGAGTTGAGTTGAAATTAGTAATCATGTTCAGAGTCATCA
GATTAATTAATGAGGGCTAGTACCTGGTTGGTTAAGTTCCAGTGGAGATGTTCA
CAGAGGTGTGTGTAAAGTGCTGCTTTATTCAACATTTTTATCATAGTGACTTTGGA
AAAGTCAGATATGTGACTTTGTTCTGAGATGCCCTATATTAGGAAAGAGCCAGAG-
GGAG
CGAGATGTTTTTGAGAGCACAGCGACCAAGTTTGAGTGACCTGAGAGAGAA
GTGGCAGAATCTAGTACACACACAAAGAATATTAGTGAGGTATTTTTACAAGATG
CAGAGAAAGAAGCTCTAAGGCAT
GGAAG
AAAATGGAAAACTACATACCTA
ATTTGGATGGGTGGACAGGAATTAAATAAGACTTC
CAAAGCACAAAGCATTCGGGGGAAATTATACAAGTGGTCTATTATTAAATTGA
GGATTTTGAGTGTAATACATACACTATGAAATTATATTCAGCAGCAGACAAATTCTCT
GACCTCATTGAATATTAAATTCCCAAAAAATGTTTTTCCACCAATTAGGAAGAAAGAA
ATTAGTTTCTACTAAGTGAATTTTCCCTTTAAATTACAATTTAAAAATATAT
GTCGGGAAG
GATCTTTTAAGGTGCTCTTTATTATTAAAAATCCACTATCTTTTTGTAT
GGTGACAATAATGGTAGCTACCGGGGCAATAGGTTGACACCTTCCACAGG
GACTATAAGCTCGACCGTGATTAATGCAGTCCAGAAGGAGACTAACATCCC
ACTTTTCTCTACAGCCTATGTTGGCAACGGGTCTGAGCTTCTACATTACTAC
AGTATAATGGCACCACATGAGGAGACTTCTACACATAAAAATTTGTGTAATATATTAAA
TATGTTATTTGGAAACAGAATCCTAGCGAGACACTGGTAACAAAACACCATGTT
AGCTATTATTATTCTACTATGTTATGATATGATGTTCTACAAATATTATTTCT
GTACACCATACCTCAAAAAATGTTGACACCTCTCGTTTACCCATAGTAAC
ATTATTTTAAAGTAAATCATCAAAAAAGGAAG
TTTATATCCTTTATTATA
TTATACCTAAAGGTGGTTAGGAGTTTTGAGGTGGCT
GGAAG
GGATGGAGACA
GACGGATCTTGGCCAAGCCCGCCCTTCTCTCAAGAGAGCTTCTCAGAGATCT
GGAGGTGTCTCTCATAGTAAAGGTGTTATTGTTACCACCAAGATTAAAATGGAA
AAAAGATGCGGCCCTCTGTCACTGAGGGGTTGACGTGACAGAGCTCAGTG
AGCAAGAAGACTGTCAGAGC
ATGAGCGCCAAGTCAGACACCATTAGGAGAT
TATTGGAGCTCCTT

TAGAAGTCAGCATGAGTTCCAACAGAAACTGGACCTGCAAGGTCAGCCCATC
AACTTGACAGGGAAGG
TCAGATTGGCAGAGAAATGGTAGTAAGTGGGATGAGATATTTTGGACAGCACCAGAAGATTAC
CAGGATGAGTTGGAGTTGAGTTGAAATTAGTAATCATGTTCAGAGTCATCA
GATTAATTAATGAGGGCTAGTACCTGGTTGGTTAAGTTCCAGTGGAGATGTTCA
CAGAGGTGTGTGTAAAGTGCTGCTTTATTCAACATTTTTATCATAGTGACTTTGGA
AAAGTCAGATATGTGACTTTGTTCTGAGATGCCCTATATTAGGAAAGAGCCAGAG-