

<i>Patient number</i>	<i>Id</i>	<i>Tumor</i>	<i>Experiment</i>	<i>Passage</i>
Patient 1	*BT130	GBM	Microarray	12
Patient 2	*BT132	GBM	Microarray	12
Patient 3	GBM6	GBM	mRNA and Fc, CTL, TGF β	8
Patient 4	MNBT110	GBM	mRNA, Fc, Westerns, CTL	14
Patient 5	MNBT112	Epd	CTL, TGF β	12
Patient 6	MNBT113	GBM	mRNA, Fc Western, TGF β	14
Patient 7	MNBT 124	GBM	mRNA, Fc	15
Patient 8	^MNBT 131	GBM	CMV, Fc	3
Patient 9	^MNBT 145	GBM	CMV, Fc	3
Patient 10	^MNBT 146	GBM	CMV, Fc	3

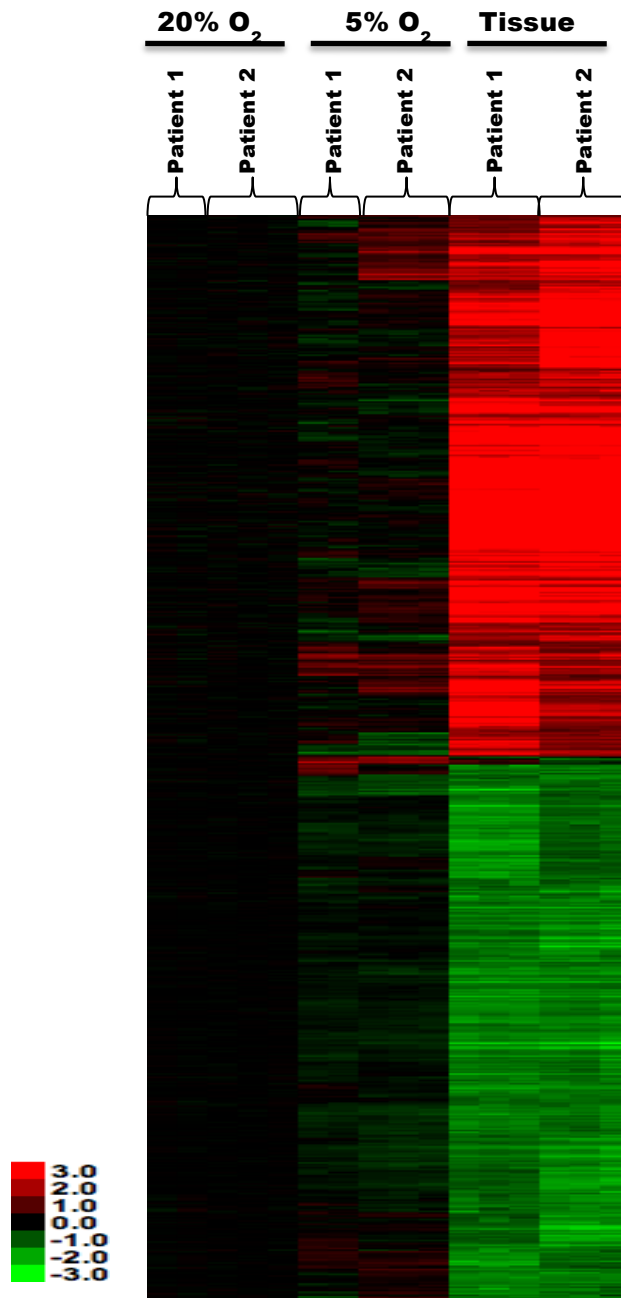
Supplementary table 1. List of tumor identification, patient information, the experiment in which the tumor was used, and passage number of the cells when the experiment was conducted. * Indicates that the tumors were resected at the Mayo Clinic, Rochester MN; others were resected at the University of Minnesota Medical Center, Fairview. ^ Indicates the cells were cultured in 5 or 20 percent oxygen directly from a surgically obtained single cell suspension; all other samples were initially cultured in 20 percent oxygen. GBM=Glioblastoma multiforme, Epd = Ependymoma, Fc = flow cytometry, mRNA = quantitative RT-PCR, Western = western immunoblot, TGF β = TGF β 1 ELISA, CTL = Cytotoxic T lymphocyte cytotoxicity assay, CMV = CMV pp65 stimulated interferon gamma assay.

<i>Antigen</i>	<i>Primer Sequence</i>
CD133	Forward-5-TCG TAC TCG GCT CCC TGT TG-3 Reverse 5- ATT CAC GCG GCT GTA CCA CA-3
SOX2	Forward 5- CCC CCG GCG GCA ATA GCA-3 Reverse 5-TCG GCG CCG GGG AGA TAC AT-3
NESTIN	Forward 5-AGG TAG AGG AGC TGG CAA GGC GAC-3 Reverse 5-TTT TCA GTA GCC CGC AGC CG-3
EPHA2	Forward 5-CTG GCC TTC CAG GAT ATC GG-3 Reverse 5-TGC ACA GTG CAT ACG GGG CT-3
IL13Ra2	Forward 5-CTG ATA AGC ACA ACA TTT GGC TCT-3 Reverse 5-TGA TGG TCT TCC ATG TTT CAC TAC C-3
HER2/Neu	Forward 5-CTG CAG CTT CGA AGC CTC ACA A-3 Reverse 5-ATG GCA GCA GTC AGT GGG CAG T-3
GAPDH	Forward 5-GTC GGA GTC AAC GGA TTT GGT-3 Reverse 5-GGG ATT TCC ATT GAT GAC AAG CT-3

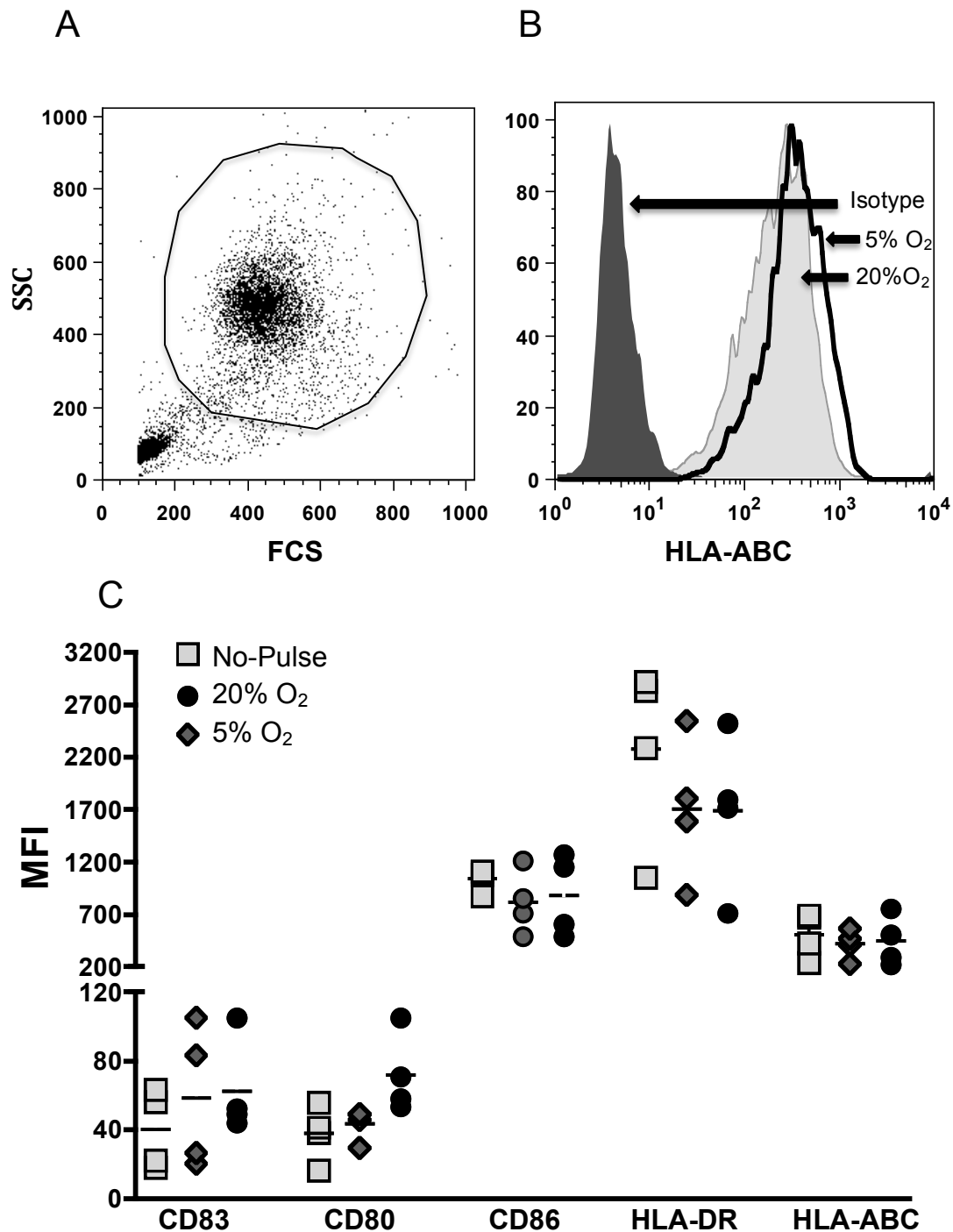
Supplementary table 2. List of primers used to determine mRNA expression levels.

<i>Donor Number</i>	<i>Experiment (s)</i>	<i>Figure(s)</i>
Donor 1	CTL, Co-Stimulatory	3, S2
Donor 2	CTL	3
Donor 3	CTL, Antigen uptake	3, 4
Donor 4	CMV	4,S3
Donor 5	CMV	4
Donor 6	Co-Stimulatory	S2

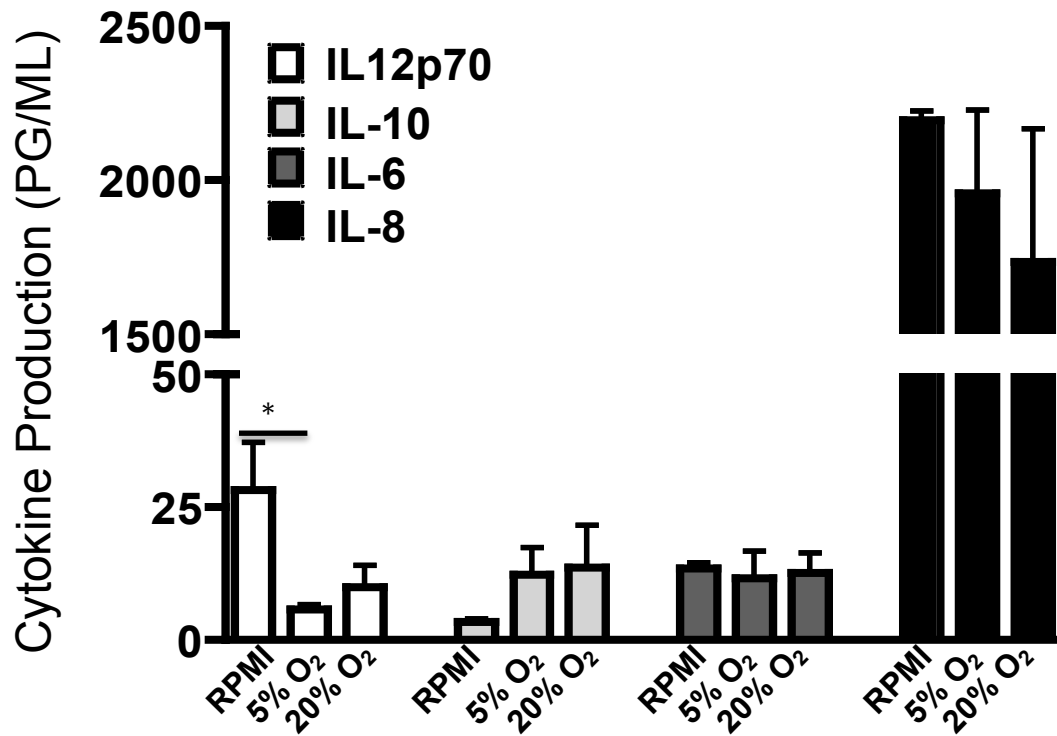
Supplementary table 3. Table of healthy leukocyte donors and the experiment in which the PBMCs were used. CMV sera-positive or sera-negative donor cells were obtained from the Red Cross, Minneapolis, MN. Co-stimulatory = expression of DC maturation markers measured by flow cytometry, CTL = Cytotoxic T lymphocyte cytotoxicity assay, CMV = CMV pp65 stimulated interferon gamma assay.



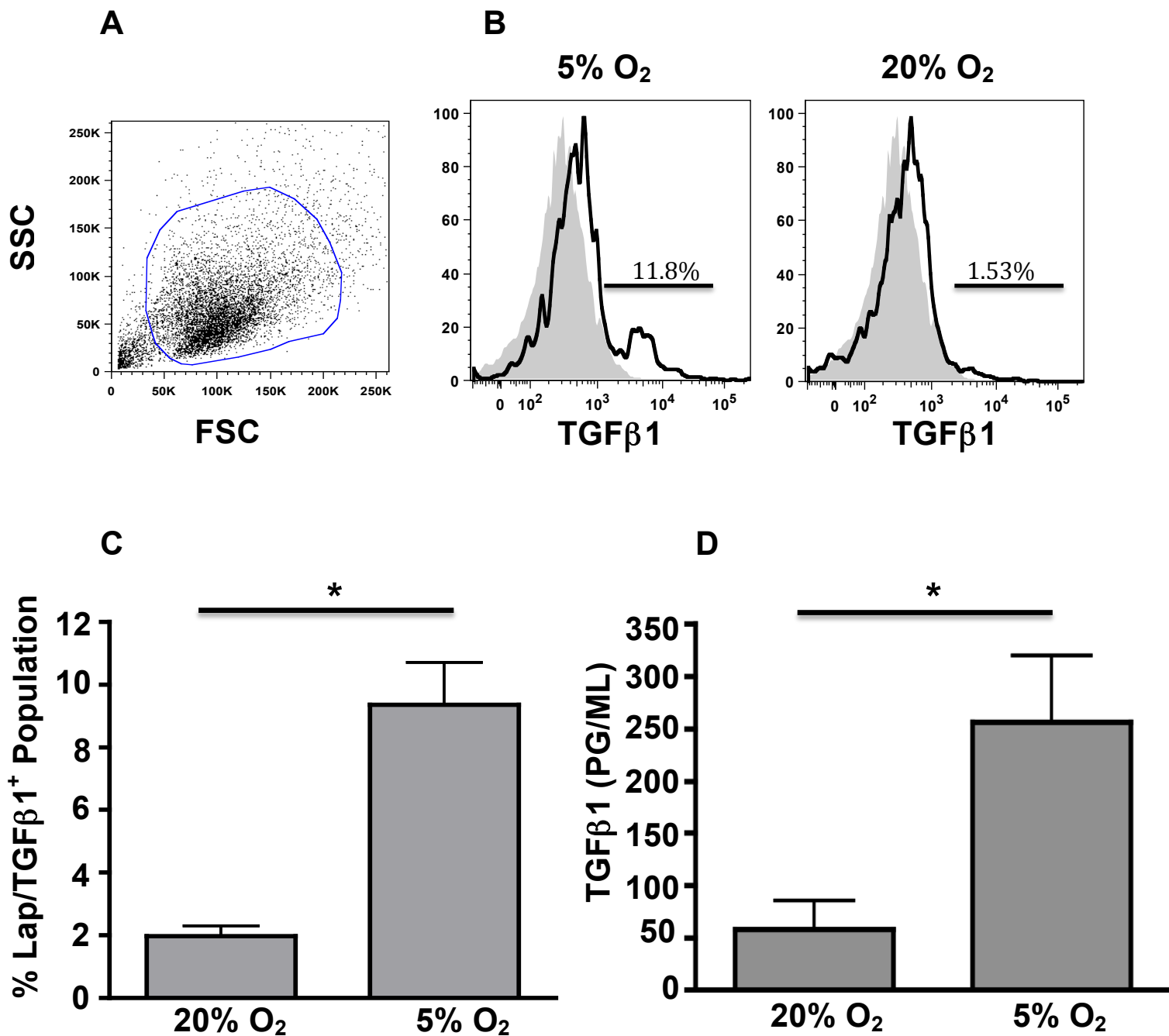
Supplementary figure S1. Oxygen-dependent gene expression compared with tumor *in situ*. Primary glioma cells cultured at 20% and 5% O₂ as well as parental tumors for two glioblastomas were analyzed by microarray. Heat maps showing log base 2 transformed data relative to the 20% O₂ state for each set of experiment for genes with p-value < 0.001 and a fold change greater than 3 for the comparison of 20% and tumor. Shown is 3,330 genes that were significantly different in expression between the tumor and cells cultured at 20% O₂.



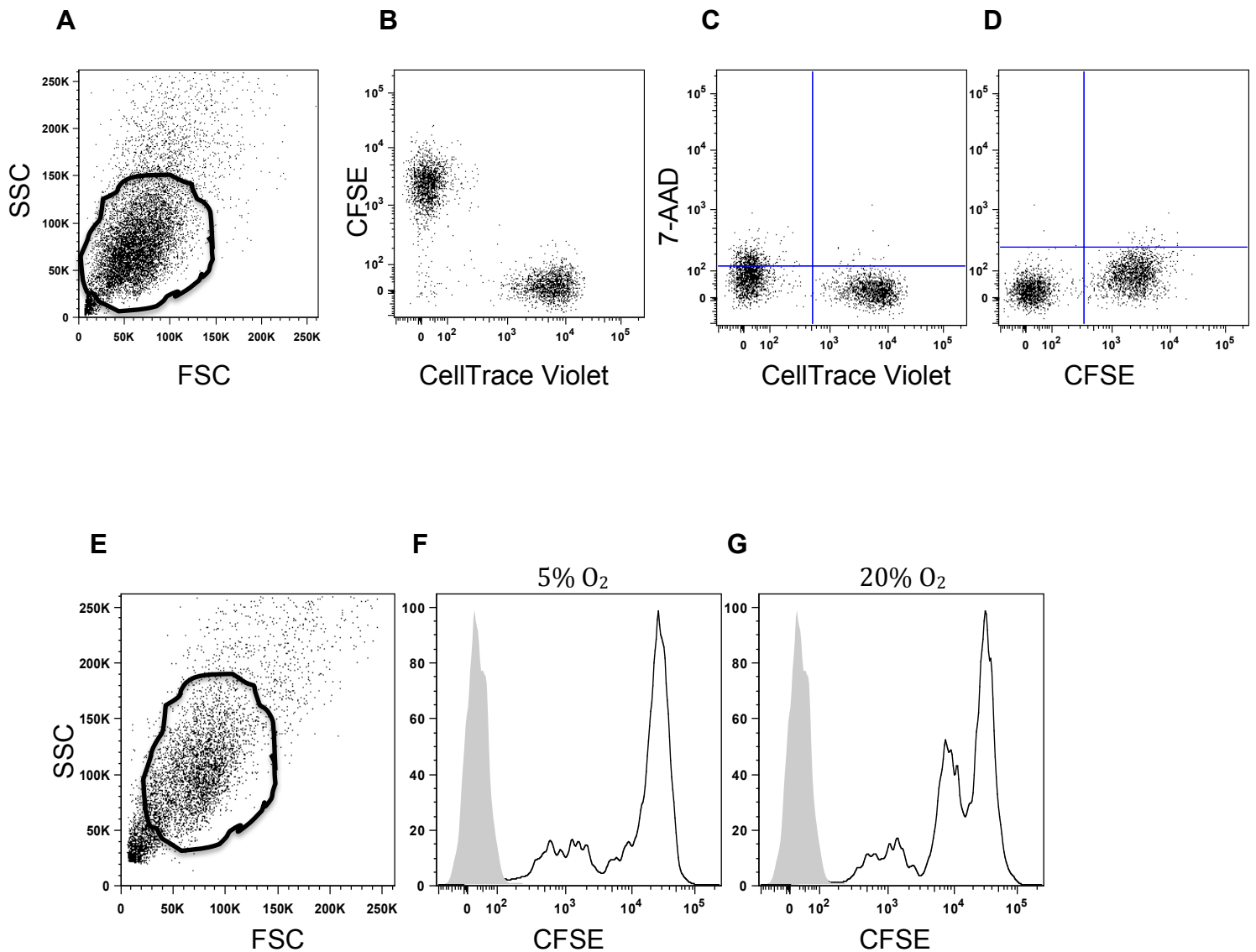
Supplementary figure S2. Increase in CD8 T cell priming is not due to an increase in these co-stimulatory or MHC molecules. To investigate if lysates derived in 5% O₂ culture altered co-stimulatory molecule expression, DCs were pulsed with tumor lysates derived in either 5% or 20% O₂, then matured. **A**, Representative DC gating strategy used in all experiments. DCs were gated from the live population, **B**, representative histogram used to calculate the mean fluorescent intensity (MFI) for CD80, CD83, CD86, HLA-DR, and HLA-ABC expression. **C**, In four separate experiments, there was no significant differences in expression of these markers between the 5% and 20% O₂ lysates (n=four separate experiments on two donors).



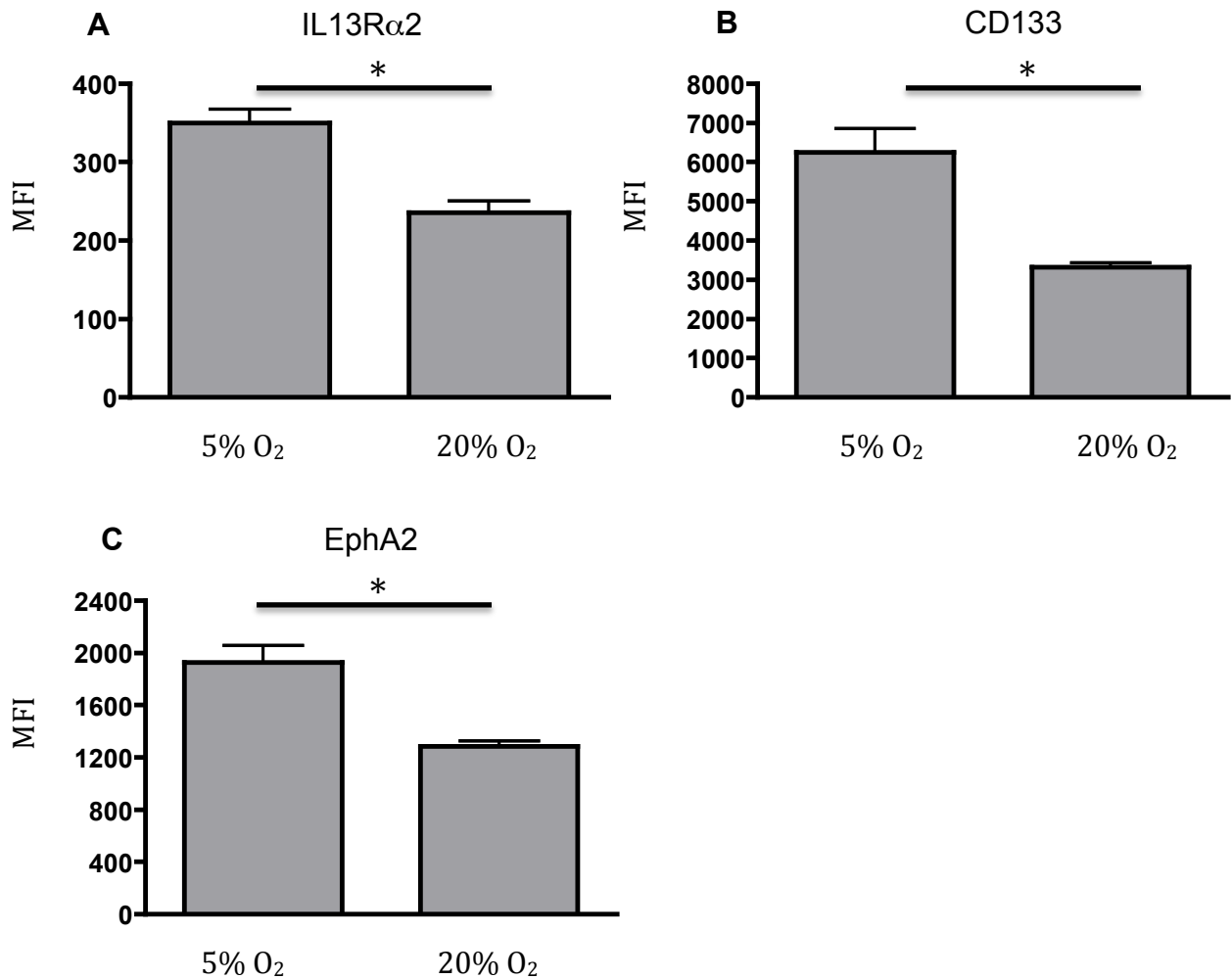
Supplementary figure S3. Cytokine production by dendritic cells pulsed with tumor lysates. Immature dendritic cells were pulsed with tumor lysates derived in 5% or 20% O₂, matured and analyzed for cytokine production by bead array (n=3 different tumor lysates, DCs were derived by donor 4). Error bars, ± SEM. * $P < 0.05$.



*Supplementary figure S4. Gliomas cultured in 5% O₂ secreted more TGFβ1. A, Resected gliomas from patients 8, 9, and 10 were cultured in 5% or 20% O₂ and phenotyped for LAP (TGFβ1) complex by flow cytometry. B, Overlay of isotype (solid) and stained (open) samples. In a separate experiment, cells were cultured for 72 h, supernatants were collected and analyzed for TGFβ1 secretion. Samples were run in triplicate (three separate patient samples were evaluated). Error bars, ± SEM. * *P* < 0.05.*



Supplementary figure S5. Representative labeling of tumor cells used as target cells for CTL or to make lysates for DC uptake. Glioma cells cultured in 5% or 20% O₂ were labeled with CellTrace™ Violet or CFSE, respectively. **A**, FSCxSSC gate. **B**, After the cells are combined in a single solution, gating demonstrates the ability separate cells cultured in 5% O₂ (CellTrace Violet) and 20% O₂ (CFSE). **C-D**, minimal background cell death is caused by labeling as measured by 7AAD staining. **E**, FSCxSSC gate. In some experiments glioma cells were cultured in 5% or 20% O₂ and labeled with CFSE alone. **F-G**, no appreciable difference in the labeling efficiency between cells cultured in 5% or 20% O₂.



Supplementary figure S6. Gliomas cultured in 5% O₂ have increased tumor antigens expression. Primary tumors directly placed in either 20% or 5% O₂ following resection where phenotyped for IL13R α 2, CD133, or EphA2 by flow cytometry (n=3 different tumors). MFI, mean fluorescent intensity. Error bars, \pm SEM. * $P < 0.05$.

Supplementary Materials and Methods

Microarray: Total RNA was isolated from snap frozen tissue at time of resection, and from cells grown in 5% and 20% O₂ using the RNEasy Plus Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Quality control was performed with an Agilent 2100 Bioanalyzer (Santa Clara, CA.). Whole-Genome Gene Expression DASL Assay (Illumina, San Diego, CA) was used to analyze expression levels of 18,401 genes. Briefly, biotinylated cDNA was synthesized from 100 ng of total RNA from each sample using mixed poly-T and random nonamer primers. Biotinylated cDNA was hybridized to assay oligonucleotides, which were subsequently bound to streptavidin-conjugated paramagnetic particles. Oligos were extended and ligated, creating templates that were amplified with random, fluorescent primers. The fluorescently labeled products were bound to 24,631 oligonucleotide probes contained on each microarray of a Illumina HumanRef-8_V1 Expression Bead Chip, and analyzed with laser confocal microscopy. Three technical replicates were analyzed for each condition for patient 2 and tissue from patient 1. Two technical replicates were performed for cell lines derived from patient 1.

Array data were analyzed for quality control as previously described (1), quantile normalized, and multiple probes for each gene were averaged. For each set of experiments (tumor, 20% and 5% oxygen) each experimental value was divided by the average of the 20% O₂ culture to determine the differences between states independent of the differences between the different tumors. Two group t-tests as well as the average fold change were used to determine differentially

expressed genes. Full microarray data was deposited at gene expression omnibus (GEO). The following link has been created to allow review of record GSE28316:

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ttedrykyisskglq&acc=GSE28316>

TGFβ1 ELISA: 1×10^6 cells were seeded in 5% or 20% O₂. Three days later, supernatant was centrifuged to remove any cells. 50 μl of supernatants were plated on a pre-coated ELISA plate (R&D Systems) and assayed according to the manufacturer's instructions. Briefly, 50 μl of supernatants were added in triplicate to pre-coated wells and incubated for 2 hrs. Wells were washed 4 times; 100 μl conjugate was added to each well and incubated for an additional 2 hrs. Following incubation, wells were washed 4 times, 100 μl of substrate was added and incubated for 30 min in the dark, pulsed with stop solution and read at 450 nm.

Western immunoblot analysis: Freshly harvested tumor cells were lysed with immunoprecipitation wash buffer containing 1% Triton X-100 and sonicated. Lysates were solubilized in Laemmli loading buffer (2% SDS, 10% glycerol, 10% 2-mercaptoethanol, 0.002% bromphenol blue, 0.0675 M Tris HCl), incubated at 95° C, and separated on 6% polyacrylamide gels. The protein was then transferred to nitrocellulose membranes, blocked with 5% milk in TBS containing 0.1% Tween 20 (TBS-T). CD133 (Abcam), IL13Rα2 (SantaCruz Biotechnology),

β -actin (SantaCruz Biotechnology) antibodies were incubated for 2 hours at room temperature. After three 5-minute washings with TBS-T, the membranes were incubated in horseradish peroxidase-labeled secondary antibodies (IQFCS) for 1.5 hours at room temperature, washed, and detected using a pico chemiluminescence kit (Thermo Scientific/Pierce) and developed. The autoradiographic film was scanned and subjected to density quantification with Alpha View[®] software.

Supplementary References

1. Sarver AL. Toward understanding the informatics and statistical aspects of micro-RNA profiling. *J Cardiovasc Transl Res*; 3: 204-11.