Supplemental Material and Methods

Flow Cytometry Staining

In general, 10^6 cells were stained in round bottom 96 well plates. Surface antibodies were diluted with staining buffer (1% FBS, 1mM EDTA, and 0.02% NaN3 in PBS) into cocktails containing Fc block (purified anti-mouse CD16/32, BD Pharmingen, San Diego, CA) and added to cells at 45µl per sample. For intracellular stains cells were permeabilized using an ebioscience kit, according to the manufacturer's protocol. For mouse studies CD45-PB (BioLegend), CD4-BV711 (BioLegend), CD25-APC-Cy7 (BD), FOXP3-FITC (ebioscience), F4/80-APC (BioLegend), CD44-PE-Cy5 (BD), CD69-FITC (BD), CD11b-BV711 (BioLegend), I-A/I-E (MHCII)-APCCy7 (BioLegend), CD19-BV785 (BioLegend), CD80-PerCP-Cy5.5 (BioLegend), CD83-PE (ebioscience), CD11c-FITC (ebioscience), CD8-AF700 (BioLegend), and IFNg-APC (BD) were used. For canine studies CD4-PB clone YKIX302.9 (AbD Serotec), CD8-AF700 clone YCATE55.9 (AbD Serotec), CD25-PE clone ACT-1 (Dako), CD3-FITC clone CD3-12 (AbD Serotec), CD79a-PerCP-Cy5.5 clone HM47 (ebioscience), and FOXP3-AF647 clone FJK-16S (ebioscience) were used. For all studies cell viability was assessed using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen), according to the manufacturer's protocol.

RNA isolation and cDNA synthesis

Total RNA from mouse tissues was isolated using RNeasy mini kit (Qiagen) with mini spin columns. RNA was reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Life Technologies) containing RT buffer, random primers, reverse transcriptase and RNAse inhibitor. RNA was transcribed for 60 min at 37°C, for 5 min at 95°C,

and then for 5 min at 4°C using AB Veriti Therma Cyler (Applied Biosystems) with 96 Well Aluminum Sample Block Module.

Quantitative Real Time PCR (qRT-PCR)

Validated primers for genes of interest were purchased from Qiagen and samples were run in triplicate. mRNA levels were calculated using the comparative threshold cycle method (Ct). Threshold cycle (Ct) values for the housekeeping gene (GAPDH) and for the genes of interest were determined and the difference between the Ct values of each gene of interest and the average GAPDH Ct was calculated (ΔCt). Differences in ΔCt (ΔΔCt) of genes of interest in treatment groups were normalized to indicated groups as shown in the equation below.

$$\Delta\Delta Ct = \Delta Ct(\text{Sample}) - \Delta Ct(\text{average of control group})$$

RT-PCR data are presented as fold change expression = $2^{-\Delta\Delta Ct}$.

Immunofluorescence

FFPE slides were routinely deparaffinized and rehydrated. Samples were submerged in target retrieval solution (Dako) and boiled in a vegetable steamer for 20 minutes. Samples were then blocked with 10% normal donkey serum and 1% Fc blocker (Miltenyi biotec) for 30 minutes. FoxP3 antibody (eBioscience; Clone FJK-16s) at 1:200 dilution factor or IDO antibody (Biolegend: Clone mIDO-48) at a 1:100 dilution factor was applied for an overnight incubation at 4°c in a humidified chamber. Slides were then washed and incubated at 1:500 dilution factor with a donkey anti-rat IgG-AF594 secondary antibody (life technologies) for 1 hour. Samples were washed and mounted with Vectashield Hard Set Mounting Medium with DAPI (Vector Laboratories). Slides were viewed and digital images taken with an inverted fluorescent microscope (EVOS, life technologies). Total nuclei (DAPI) and total positive staining cells were

quantified using ImageJ image analysis software. For FoxP3 entire high powered fields were analyzed and for IDO glandular formations were analyzed.

Immunohistochemistry

Immunohistochemistry was performed on frozen tissue sections mounted on charged slides and air-dried overnight. Slides were acetone fixed for four minutes, air-dried, then quenched for 10 minutes in 0.1M Phosphate Buffered Saline, pH 7.4 (PBS) with 0.3% hydrogen peroxide and 0.1% sodium azide. Slides were rinsed in PBS, then treated with 10% normal horse serum in PBS for 20 minutes. Without rinsing, the CD8▪ antibody diluted 1:10 in PBS was applied and incubated for 1 hour. Slides were rinsed, and biotinylated anti-mouse antibody diluted 1:500 in PBS was applied for 20 minutes. Slides were rinsed, and streptavidin-horseradish peroxidase diluted 1:500 in PBS was applied for 20 minutes. The detection was visualized with Vector's NovaRed, per manufacturer's instructions. All incubations occurred at room temperature. Sections were counterstained with Mayer's Hematoxylin, air dried and coverslipped.

Kynurenine / Tryptophan ratio

Serum from treated and untreated mice or canines was analyzed using a 6 minute isocratic run of 95:5 water:acetonitrile (both with 0.1% formic acid) on a Synergi 2.5um Polar-RP, 50x3mm column (Part No. 00B-4371-Y0). Tryptophan and Kynurenine eluted at 3.77 and 2.5 minutes respectively with a flow rate of 0.3mL/min.