**Supplemental Methods**

**Antibodies and reagents**

Purified antibodies against argininosuccinate synthetase (ASS-1, clone 25), arginase I (19), iNOS (54), CD3 (1452C11), CD8 (53-6.7), CD28 (37.51), CD11b (M1/70), CD45.2 (104), Gr-1 (RB6-8C5), Ly6C (AL-21), and Ly6G (1A8) were obtained from Becton Dickinson Biosciences (BD Biosciences, San Jose, CA). IFN- (XMG1.2), CD25 (PC61.5), CD69 (H1-2F3), CD45.1 (A-20), CD4 (GK1.5) antibodies were purchased from eBioscience (San Diego, CA). Anti-β-actin antibody (AC-74) was obtained from Sigma-Aldrich (St. Louis, MO). Antibodies against Glut1, argininosuccinate lyase (ASL), cyclin D3, and cdk4 were from Santa Cruz biotechnologies (Santa Cruz, CA). L-NG-Monomethylarginine (L-NMMA) and Nω-hydroxy-nor-Arginine (NN) were obtained from EMD Millipore (Calbiochem, Gibbstown, NJ).

**Flow Cytometry**

Cells were stained with specific antibodies and analyzed using a Gallios Flow Cytometer (Beckman Coulter, Miami, FL). For intracellular detection of IL-2 or IFN, activated-T-cells (24 and 72 hours, respectively) were cultured in presence of Golgi Stop (Becton Dickinson), phorbol-myristate acetate (PMA, 750 ng mL-1) and ionomycin (50 µg mL-1) for 6 hours, followed by intracellular staining using Cytofix/Cytoperm staining buffer (Becton Dickinson). Proliferation of T-cells *in vitro* was measured using the intracellular dye carboxy-fluorescein succinimidyl ester (CFSE) (Molecular Probes, Life Technologies). T-cell proliferation *in vivo* was monitored by uptake of 5-bromo 2’deoxyuridine (BrdU).

**Cell fluorescence**

CD3+ T-cells were adhered to slides by cytospin, fixed in −20°C methanol for 10 min and blocked with 5% BSA in PBS. Slides were incubated with Mitotracker Green FM (Invitrogen) at 100 nM per manufacturer’s instructions, followed by nuclear staining with DAPI. Images were captured at equal exposure using a Nikon Eclipse E400 upright fluorescence microscope equipped with EXI aqua camera (Qimaging), motorized Z-axis, and SlideBook5 acquisition /deconvolution software (Intelligent Imaging Innovations, Inc). A series of three-dimensional images of each individual picture were deconvoluted into one two-dimensional picture and resolved by adjusting the signal cut-off to near maximal intensity to increase resolution. Quantification of voxel/cell was performed by utilizing Mask analysis included in SlideBook5 software (Intelligent Imaging Innovations).

**Statistical Analysis**

Statistical analyses were carried in SAS 9.3 (SAS Institute, Cary, NC). Tests were conducted at 5% significance level. Experimental groups differences of endpoints were assessed by ANOVA with the Satterthwaite correction for unequal variances using the MIXED procedure. Means comparisons were achieved using the Tukey procedure for all comparisons or with the Dunnet procedure for comparisons with the control group.

**Supplemental Figure Legends**

**Supplemental Figure 1. Peg-Arg I blocks T cell proliferation and IFN production.** (A) T-cells (1x106) were CFSE-labelled and stimulated with anti-CD3 + anti-CD28 in the presence of peg-Arg I or peg-BSA (1 g/ml). Cells were stained with anti-CD3, CD4 or CD8 and percentages of proliferating cells were determined at 72 hours by flow cytometry. (B) Activated T-cells were cultured in the presence of peg-Arg I or peg-BSA (1 g/mL) for 72 hours, then tested for IFN expression by flow cytometry.

**Supplemental Figure 2. Peg-Arg I does not alter T cell percentages in naive mice.** (A-C) Mice were treated with increasing concentrations of peg-Arg I, peg-BSA or PBS every 2 days for 7 days. Spleens were isolated, RBC-lysed, and stained with anti-CD3 (A), CD4 (B), or CD8 (C).

**Supplemental Figure 3.** **Effects of peg-Arg I on T-cell glycolysis.** (A) T-cells were treated with increasing concentrations of peg-Arg I or peg-BSA for 48 hours. ECAR was measured via an extracellular flux analyser. (B) Activated T-cells cultured with 1 g/mL peg-Arg I or peg-BSA for 72 hours, after which ECAR was analyzed under basal conditions and in response to 10 mM glucose, 10 µM oligomycin and 100 mM 2-DG.

**Supplemental Figure 4.** **ASS-1 Silencing in T-cells.** Activated T-cells (2x105)were treated with ASS-1 or non-targeting control siRNA (2 M) for 48 hours. Whole-cell lysates were obtained and the expression of ASS-1 was tested by Western blotting.

**Supplemental Figure 5. Asparaginase depletion induces MDSC accumulation.** Mice were injected i.p. with 3000 U/mice peg-Asparaginase every 2 days for 7 days. Then CD11b+ Gr1+ cells were tested in the spleen by flow cytometry.

**Supplemental Figure 6. Peg-Arg I increases arginase activity in CD11b+ Gr1+ cells**. Mice were injected with various concentrations of peg-Arg I or peg-BSA for 7 days, then CD11b+ Gr1+ cells were isolated and arginase activity was determined.